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LEE ELLIS MILES

September 25, 1890–May 10, 1941

DAVID C. NEAL

Dr. Lee Ellis Miles, Plant Pathologist of the Mississippi Agricultural Experiment Station and Agent of the Division of Cotton and Other Fiber Crops and Diseases, U. S. Department of Agriculture, died suddenly of a heart attack on May 10, 1941, at Greenville, Mississippi. At the time of his death he was collecting field data on cotton and oat diseases in the Mississippi Delta region. He was the son of Charles and Sarah Jane Acres Miles, Irish and Welsh parents, and was born in Rockville, Indiana, September 25, 1890. He was valedictorian of the graduating class of Rockville High School in 1910, and was graduated from Wabash College with the A.B. degree in 1914. For high scholastic ability in his junior year at Wabash, he was elected to Phi Beta Kappa. He was a member of Lamba Chi Alpha social fraternity. Dr. Miles served in the Indiana National Guard in 1914 and, during the World War I, he was attached to the 25th Machine Gunners' Battalion, U. S. Army.

In 1917, the University of Illinois awarded him a fellowship in plant physiology and, in 1920, he received the degree of Ph.D. from the same institution. His doctorate dissertation was "Leaf Spots of the American Elm."

During the summers of 1919–20 he was employed by the Bureau of Plant Industry, U. S. Department of Agriculture, in Barberry eradication and White Pine Blister Rust Control in the States of Illinois, Michigan, Wisconsin, and Tennessee.

From 1920 to 1922, Dr. Miles served as plant pathologist for the Mississippi State Plant Board. He was in charge of eradication of diseases of sweet potatoes, sugar-cane mosaic, etc., plant-disease inspection of nurseries, and was a member of the advisory board on plant-disease control. In 1922, he resigned his position in Mississippi to become pathologist of the Alabama Agricultural Experiment Station and Professor of Plant Pathology at the Alabama Polytechnic Institute, Auburn, Alabama. During his residence in Auburn he was married to Miss Eunice Rebecca Stodghill, who survives him together with two daughters, Lallah Jane and Mary Martha Miles. In 1927, Dr. Miles accepted the position of Assistant Plant Pathologist with the Experiment Station and Assistant Professor of Plant Pathology at State College of Washington, Pullman, Washington. He remained in this position only one year and returned to Mississippi in the summer of 1928, where he served as Plant Pathologist of the Mississippi Agricultural Experiment Station, State College, Mississippi, to the time of his death. At the Mississippi station, he became actively interested in the cotton-disease investigations sponsored by the Cotton Disease Council and conducted in cooperation with the Bureau of Plant Industry, U. S. Department of Agriculture, and made

LEE ELLIS MILES

several scientific contributions in this field. He investigated particularly the seedling diseases of cotton, cotton seed treatment, the effect of period and type of storage following treatment, and varietal resistance of cotton to fusarium wilt and root-knot nematode. He also was the first to discover verticillium wilt of cotton and downy mildew of oats in Mississippi. He spent the summer of 1940 at Harvard University in taxonomic research on this downy mildew fungus.

He was a student of mycology, two of his noteworthy publications being: "The Rusts of Mississippi," and "The Ascomycetes of Mississippi," published during the years 1933–35. He knew the fungi and their host plants well; and in conducting research in applied plant pathology, he proceeded not only with scientific vision but with the practical aspects of plant-disease control as the uppermost objective. He enjoyed botanical field trips, botanical collecting, and athletic sports (particularly football, basketball, and track), and took an active part in scientific meetings. He served the Southern Section of The American Phytopathological Society as Secretary from 1923–25; President, 1930–31; and as member of the Council of the American Phytopathological Society 1932–33. He was a life member of The American Phytopathological Society, member of the Botanical Society of America, member of the Executive Committee of the Cotton Disease Council, Fellow of American Association for the Advancement of Science, and member of Sigma Xi.

A forceful speaker and endowed with a dry sense of humor, Dr. Miles always commanded attention and respect whenever called upon to make impromptu remarks or to address a scientific meeting or other assemblage. A strong personality was Dr. Miles, devoted to his family, loyal to his friends, and sympathetic and tolerant toward the views of others. He lived a full and fruitful life, which endeared him to a host of friends. Regardless of whether it was the college associate, the merchant, or family doctor who accompanied him on deep-sea fishing trips, the farmer or plant grower who discussed his plant-disease problem with him in the field, or the co-worker who collaborated with him on disease surveys or at scientific meetings, the lives of all of these acquaintances are richer for having known him.

His passing marks a distinct loss to plant pathology, to southern agriculture in particular, and to Mississippi, his adopted State, whose citizens he served with distinction for many years.

Dr. Miles conducted research and published numerous articles in varied fields of plant pathology including diseases of cereals, cotton, nursery and ornamental plants, pecans, truck crops, and sugar cane. A list of his publications together with those of joint authorship follows:

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DIFFERENCE IN pH RELATIONS OF SOME PATHOGENICALLY VARIABLE STRAINS OF TOMATO FUSARIUM

FREDERICK L. WELLMAN

(Accepted for publication July 22, 1941)

INTRODUCTION

The well-known tomato wilt caused by *Fusarium bulbigenum* var. *lycopersici* (Brushi) Wr. and R., has been an important disease for many years. Strains of tomato that are wilt-resistant under ordinary field conditions have been bred and selected by numerous workers. At times, however, these varieties are more severely damaged by *Fusarium* than might be expected; a fact that may be due in part to the presence of fungus strains of increased virulence. More highly wilt-resistant varieties of tomato are being developed (1, 4), and, in the course of such work done at the U. S. Horticultural Station, Beltsville, Md., studies have been made of the relative virulence of a number of strains of the causal organism.

A considerable range of pathogenicity (11) has been shown to occur in strains[1] of the tomato wilt *Fusarium*, varying from those of mild type, producing little damage, through those causing increased disease, to a highly virulent type. Attention also has been given (12) to the direction and rapidity of variation. While studying variant *Fusarium* strains in culture and pathogenically, contrasts were seen to be so great as to indicate the probability of marked divergence in physiology of the strains *in vitro*. These observations were pursued further, and one phase of the problem included a study of certain pH relationships. The research here reported does not represent an exhaustive study of hydrogen-ion relationships of the tomato-wilt *Fusarium*, since the results were considered first of all as an addition to the data on the divergence of strains of the organism. It is believed, however, that they may also be of interest in connection with the complex problem of relative virulence in variant isolates.

MATERIAL AND METHODS

A total of 79 different isolates or strains of *Fusarium bulbigenum* var. *lycopersici* were used in these studies. One series of 28 strains had been previously studied and described (11) and its members were known as to pathological and cultural histories. They varied pathogenically from extremely virulent through intermediate types to very mild when tested on seedlings of Bonny Best and Marglobe tomato and, culturally, from the class bearing raised, white, woolly mats through intermediate types to the appressed class characterized by dark slimy mats with little or no aerial growth. Of these strains, two were selected for intensive studies on the basis of approximately the widest divergence in pathogenicity, as already determined (11, 12). The most virulent isolate was No. 5; the mildest was No.

[1] The term "strain" refers to cultural progenies from a given isolate.

15, both of single-spore origin. These were then further "pure lined" by use of monosporic reisolation methods. With both strains, 8 monosporic isolates were made for each "cultural generation." These were grown and tested according to standard technique (10) for pathogenicity. In the case of the R5 isolates, 6 "generations" were grown, and in each generation the culture giving highest pathogenicity was kept for reisolation purposes and the 7 remaining cultures discarded. The same method was followed for 8 generations in the case of the A15 strain, except that the colony from which lowest pathogenicity index was obtained was used for the reisolation purposes. The object of this procedure was selection of an "R" strain on the basis of highest pathogenicity and selection of an "A" strain on the basis of lowest pathogenicity. These were designated separately from their parents, namely, R5–6 and A15–8; and have been compared in detail in table 1.

TABLE 1.—*Comparison of certain characters of the R5–6 (virulent) and A15–8 (mild) strains of Fusarium bulbigenum var. lycopersici*

Points of comparison	Strain R5–6	Strain A15–8
Source of strain	6 monosporic reisolations from R5[a]	8 monosporic reisolations from A15[a]
Culture class and virulence[b]	Raised, virulence index = 15.00	Appressed, virulence index = 1.90
Aerial growth on agar[c]	Abundant, fine woolly	Scanty tufts of hyphae
" " " liquid[d]	Thick, fluffy, floating	Thin, slimy, mostly submerged
Mat texture on agar	Tender, cheesy	Tough, stringy
" " in liquid	Brittle, folded and warty	Tough, smooth
Color[e] of agar culture	White to pale lavender	Vinaceous purple to light buff
Color of rice culture	Pale persian lilac	Cinnamon
Color of liquid culture	White to pale vinaceous lilac	Tawny
Odor of agar culture	Ammoniacal	Sweetish aldehyde
Odor of liquid culture	Strong ammonia	Slightly sour, yeasty

[a] Previously described and studied (11, 12).

[b] Reference is made to prior publications (10, 11, 12) for definition of culture classes and numerical evaluation of virulence. Indexes from parallel tests on 20 plants of Bonny Best tomato: 15.00 = plants dead and collapsed; 1.90 = plants with browned vascular bundles in roots nearly up to cotyledonary node, but externally plants apparently healthy.

[c] Agar of the "differential formula" described in this paper. Grown 2 weeks on all media.

[d] Liquid used was of Tochinai formula (10).

[e] Color according to Ridgway (6).

In addition to the 30 isolates just mentioned, 49 others were secured within 2 summer seasons from field-grown Bonny Best plants that were in various stages of wilt. These latter cultures were single-spore isolates, each from a separate plant, except for 6 saltants out of these, which also were included. These cultures were all found to be well within the limits answering the description by Wollenweber and Reinking (14) of *Fusarium bulbigenum* var. *lycopersici.*

The culture medium generally used was made according to the Tochinai formula (10). As a liquid medium it had a pH of about 6.0 to 6.2. Different reactions were produced by the use of separately prepared and sterilized

dilute hydrochloric acid and sodium hydroxide solutions, mixed, after cooling, in equal quantities with double-strength culture medium. It was found that Tochinai agar did not give the most distinctive reactions between different cultural variants. By varying the components of several of the best known of the standard culture media used with fusaria, a formula was finally devised that gave the most rapid and consistent results in differential studies between R5–6 and A15–8, as well as other strains. The formula is as follows: proteose peptone 5.0 g., dihydrogen potassium phosphate 0.5 g., magnesium sulphate 0.5 g., maltose 15.0 g., ferrous sulphate 0.03 g., agar 12.0 g., water 1000.00 cc. As a liquid it had a pH value of about 5.2 to 5.4.

All glassware employed was of the "insoluble" type. Liquid cultures were grown in 100 cc. of liquid in 200 cc. small-mouthed Ehrlenmeyer flasks. Agar cultures were in Petri dishes 9.0×1.5 cm. containing approximately 20 cc. of agar. Every pH determination was made with the Beckman pH meter, standardized at frequent intervals against known buffer solutions. Except in a few cases that are mentioned, cultures were held in a dark incubator at an approximate temperature of $27.5° (\pm 1.2°)$ C.

Standard-size pieces of inoculum were taken from 21-day-old Tochinai agar plates that had been inoculated in the center. These plates were set in the center of a block on which was drawn a circle the exact diameter of the bottom half of a Petri dish containing agar. Extending at equidistant points around the periphery of the circle were markings to indicate radial lines. A sharp-edged steel tube was employed to cut disks of 5.5 mm. diameter from near center to edge along these radial lines, so that the inoculum for each cultural treatment within a test was taken as nearly as possible along one radius of mat growth. Preliminary studies showed that in apparently stable strains of *Fusarium* this technique resulted in a uniform selection of inoculum. In obviously saltating strains results of saltant action were more certain of demonstration, since it was observed that sectors occurred more frequently along radial lines. When numerous disks for inoculum were cut, supposedly at random, in an agar plate there was apt to be unconscious selection of disks on the basis of ease of handling rather than age of growth or cultural variability.

Every flask of liquid was inoculated with a single disk of inoculum. These were shaken immediately, put in the incubator, and left untouched until the desired period of growth had elapsed. In the case of mixed cultures, a half-disk of one strain matched with the half disk of another was used for inoculum. In agar experiments disks were placed with aerial growth down at the center of Petri plates, on the hardened agar surfaces, care being taken to avoid any sliding of the disk on the medium.

Each datum that refers to either pH readings or mat weights represents the mean of at least 3 determinations. Weights were secured by first pouring the contents of incubated flasks onto weighed, air-dry filter paper in a Buchner funnel attached to a low vacuum water pump. Excess liquid was then withdrawn, mats were washed with 100 cc. of cold water, which was

filtered off, and the mat and papers dried at room temperature. This room was steam-heated, and, under the local laboratory conditions, drying proceeded rapidly and approached equilibrium in about 4 days. Final weights, however, were not recorded until after drying 14 to 16 days. To correct for the weight of the inoculum air-dry weights of 9 disks from each place used as source of inoculum were obtained by dropping newly cut disks into liquid medium, shaking, filtering immediately, and drying. The average inoculum weight was subtracted from the final mat weight, so as to indicate growth on the basis of net weight. Growth of cultures was on the whole very uniform, variations being small within triplicates. This also was true of mat areas obtained on agar cultures.

In the case of growth on agar, results were recorded as areas in square centimeter of agar covered. Means were taken in millimeters of 4 radii on each colony grown in a triplicated pH series. From this a "gross" area was calculated from which the mean area of the pieces of inoculum were subtracted.

Disease evaluations were determined (10) on the susceptible and tolerant test hosts, Bonny Best and Marglobe. Numerical indexes are briefly as follows: 0 = no observable internal or external disease symptoms; 1, 2 = mild disease, discolored vasculars in base of plant up to cotyledonary node, no leaf symptoms; 3, 4, 5 = moderate disease, darkened veins in the base of stem, lower leaves wilting; 6, 7 = serious disease, dark vasculars in more than half of stem, lowest leaves wilted, leaves in central portion of plant wilting; 8, 9 = severe disease, dark vasculars extending nearly to tip, almost all leaves wilted except those at tip; 10 = very severe disease, vasculars dark full length of stem, all leaves, even at tip, wilting or dead, stem of plant upright; 11 to 15 = extreme severity, all leaves dead, stem condition ranging from first signs of collapse to complete prostration.

PROGRESSIVE pH CHANGES IN A LIQUID MEDIUM

Parallel sets of flasks of Tochinai liquid having a pH of approximately 6.1 were inoculated with R5–6 and A15–8 and placed in the incubator. Sample sets of 3 cultures were removed from these series at intervals for determinations of change both in pH and in weights of air-dry mats. Data from 5 series were taken at the following intervals: series 1, at 0, 3, 10 and 15 days; series 2, at 0, 10, 15, 20, 30 and 40 days; series 3, at 0, 1, 4, 7, 10, 20 and 32 days; series 4, at 0, 5, 12, 19, 50, 100, 120 and 150 days; and series 5, at 0, 3, 5, 8, 12, 16, 22, 27, 33, 41, 53, 60 and 67 days.

From what had been learned from previous series, the fifth was designed so that samples were taken at the best intervals to demonstrate as complete details as practicable of the critical pH and weight changes. Data from the other series differed so little from No. 5 that the latter may be taken as characteristic of all the series. Results from it are therefore the only ones to be given and are represented in figure 1, A and B.

It will be seen from the curves representing weight changes (Fig. 1, B)

that the virulent strain produced much greater mat weight than did the mild strain, and the high point was gained by the virulent strain at around 12 days' growth. It took the mild strain 22 days to reach its greatest

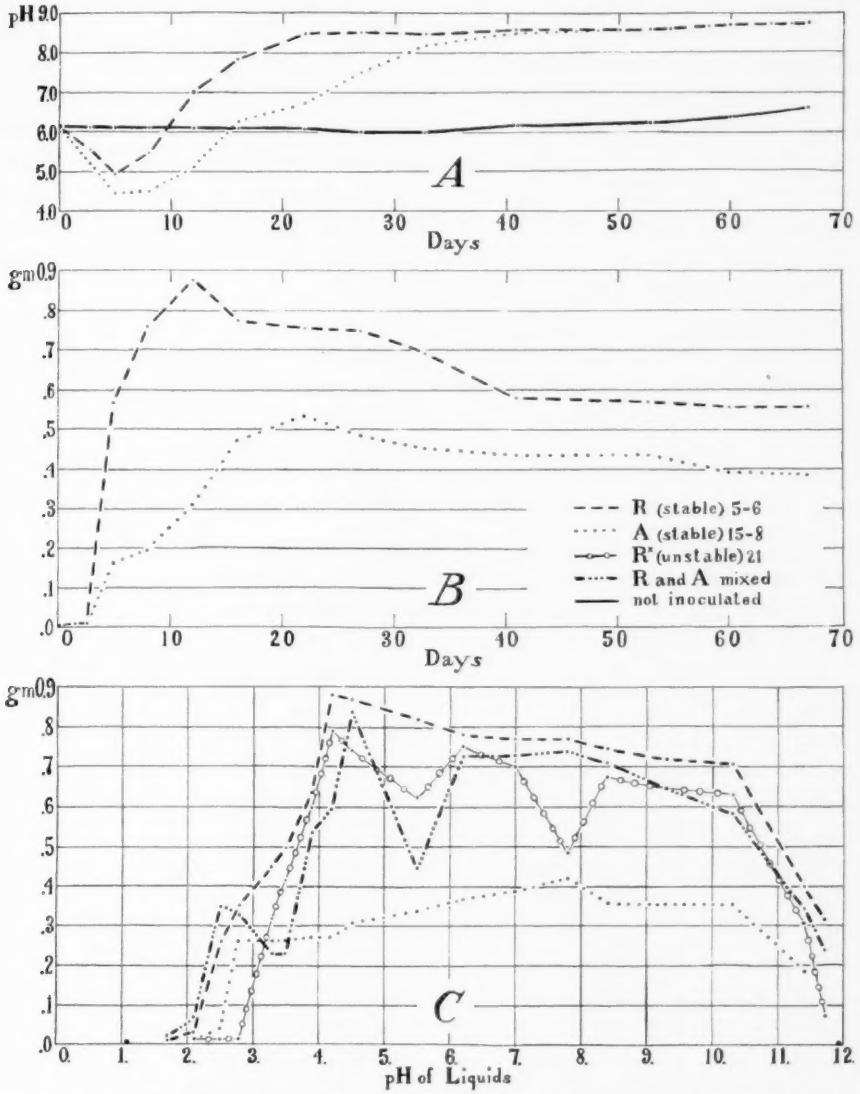

FIG. 1. Growth in liquid cultures (Tochinai formula) of pathogenically different strains of *Fusarium bulbigenum* var. *lycopersici*. A. Progressive changes in pH produced by a virulent (R5–6), and a mild (A15–8), strain of the organism. B. Progressive mat-weight determinations for the virulent and mild strains. These data taken concurrently with A and from the same cultures as those tested for pH. C. Mat weights produced after 16 days' growth in media initially adjusted to a wide range of pH.

weight. After reaching their respective high points, both strains lost some weight, apparently because of partial disintegration, or autolysis, of the mycelial mats. This loss was arrested finally, as the curves indicate. It

will be noted in figure 1, A, that immediately after either of the strains began growing acidity increased in the liquids for the first 5 days, and was most intense in cultures of the mild organism. In these cultures the pH then rose gradually until about the 40th day. It took the virulent culture about half the time required by the mild organism to go through the same course of changes.

It is interesting also to observe differences in the concurrent pH and weight changes produced by the A and R strains (Fig. 1, comparing curves shown in diagrams A and B). For R5–6, by far the greatest increase in mat weight was at the beginning of growth between $2\frac{1}{2}$ and 5 days after inoculation, during which time the liquid was becoming most acid. For A15–8, on the other hand, a comparatively rapid increase in mat weight continued in progress for around 15 days after growth in culture was well established. During this time the liquid attained its greatest acidity, then it became less acid until it approached neutrality. It required about 40 days for R5–6 cultures to reach a point where little further loss in mat weight occurred, and about 60 days for the A15–8 cultures.

GROWTH AFFECTED BY DIFFERENT pH REACTIONS OF MEDIA

Agar Medium

It should be repeated here that no attempt was made to execute a complete study of the relation of pH ranges in media to growth of strains of the tomato-wilt *Fusarium*. The main purpose was the demonstration of differences in the physiology of strains of the pathogen.

In figure 2 are represented smoothed curves giving the comparison between areas covered after 115 hours of growth at 27.5° C. on agars of different initial pH reactions by the virulent and mild strains. These data are the means of results from 3 experiments that were run within a few days of each other, and for the same number of hours in the same temperature chamber. The agars in each (handled in triplicate) were adjusted to 12 pH points from 2.2 to 11.7. Except in a very few instances in the extremely acid or alkaline agars, the curves for the individual experiments ran close to and did not cross the smoothed curves depicted in figure 2.

On agar the optimum pH ranges were much alike for strains A15–8 and R5–6. The greatest areas of growth were covered with both strains between a pH of 7.0 and 8.7. The greatest difference between these cultures on agar was seen in the comparative amounts of growth produced. Nearly 20 per cent more area was covered by the mild strain of the organism than by the virulent strain.

Liquid Medium

Studies were likewise made of mat weights produced in liquid medium adjusted to different pH readings. Results of typical experiments are represented in figure 1, C, and the data from the other series are given in tables 2, 3, and 4. There were slight divergences, but, on the whole, the virulent

R5–6 strain had an optimum at the quite acid reaction of approximately pH 4.0, while the mild A15–8 had an optimum at a definitely alkaline reaction of about pH 8.0. Extremely acid (pH 2.0 to 3.0) and extremely alkaline (pH 11.0 to 12.0) media were unfavorable to about an equal degree for both strains of the organism. When mat weights from either of the stable strains, A15–8 or R5–6, were plotted against original pH reactions of media (Fig. 1, C) the results were comparatively regular curves having single optimum points. When the data from unstable strains were handled in the same way, the curves showed considerable irregularity with more than one point of optimum growth.

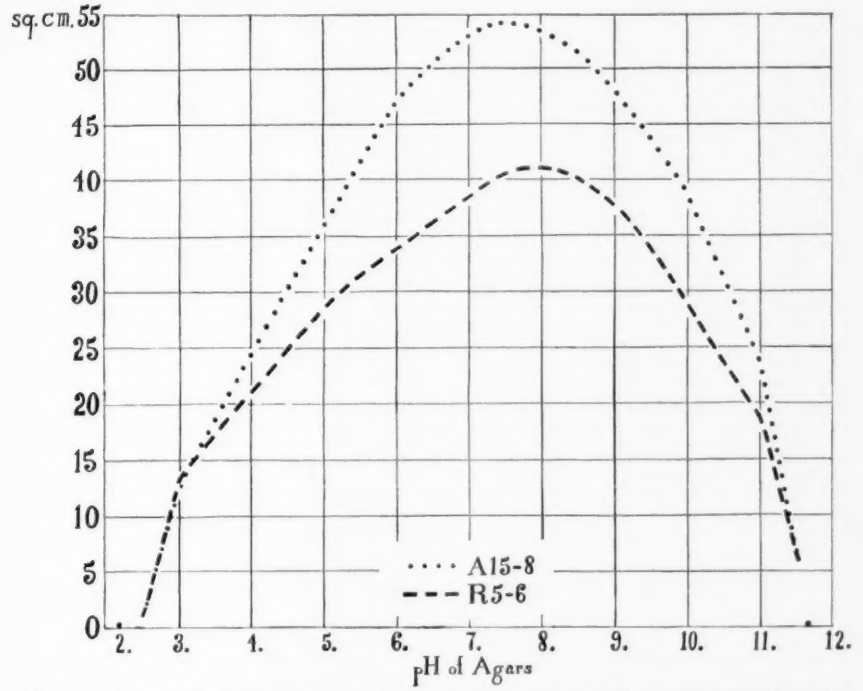

FIG. 2. Areas covered by a virulent and a mild strain of the tomato-wilt *Fusarium* on agar (Tochinai formula), adjusted at 12 points over a wide range of pH. The smoothed curves represent the mean areas covered at these points on 9 agar cultures from 3 separate series. Each series was grown 115 hours at 27.5° C.

Equal-size bits of mycelium from A15–8 and R5–6 were combined and used to inoculate a total of 4 culture series that had wide ranges of pH. After incubation, mat weights were obtained from these series, and the results (Fig. 1, C, and Table 3) gave an indication of the occurrence of more than one maximum growth point with considerably reduced growth between these rises. The general tendency seemed to be for two such maxima to develop. In addition to this tendency, seen among tests in the series inoculated with mixtures of the stable strain and mild strain, what appeared to be bimodality was found in all of 7 tests of cultures from 3 strains of the *Fusarium* known to be unstable and that were distinctly saltating at the

time they were used for inoculum. The significance of these findings in comparison with the findings of other workers on the same organism, will be examined below in the discussion.

TABLE 2.—*Growth*[a] *in Tochinai liquid medium of virulent (R5–6) and mild (A15–8) strains of Fusarium bulbigenum var. lycopersici as influenced by various initial pH reactions*[b] *of the medium*[c]

Series 1[d]			Series 3[d]		
Reaction of medium	Average mat weights		Reaction of medium	Average mat weights	
	R5–6	A15–8		R5–6	A15–8
pH	*Gm.*	*Gm.*	*pH*	*Gm.*	*Gm.*
1.0	.00	.00	3.1	.27	.17
1.7	.01	.00	3.2	.38	.18
2.1	.03	.01	3.6	.50	.18
2.8	.35	.26	4.2	**.56**	.20
3.3	.49	.27	4.7	**.57**	.21
3.8	.65	.25	6.0	**.53**	.23
4.1	**.89**	.27	6.7	**.53**	.25
4.3	**.87**	.30	7.2	.50	.29
5.2	**.83**	.29	7.7	.50	**.32**
6.1	.70	.31	8.1	.49	**.32**
6.8	.70	.34	8.4	.46	.26
7.8	.71	**.40**	9.1	.41	.24
8.6	.71	**.37**	9.7	.37	.25
10.2	.68	.35			
11.2	.41	.19			
11.7	.30	.01			
12.0	.00	.00			

[a] Growth recorded as the average weight of 3 fungal mats.
[b] Reactions regulated through use of dilute HCl and NaOH.
[c] Tochinai liquid (10).
[d] In these experiments 4 series were run: Data from series 2 are represented in figure 1, C; from series 4 presented in table 3. All series incubated in the dark at approximately 27.5° C., series 1 and 2 for 14 days; series 3 for 9 days; series 4 for 16 days.

CHANGE IN REACTION OF LIQUID CULTURES STARTED AT DIFFERENT pH LEVELS

Changes in pH of liquid media having different initial reactions were studied in parallel series inoculated with the virulent R5–6 and the mild A15–8 forms. There were 4 experiments in these studies, and in each one variations were seen between the growth changes of the mild and virulent organisms, as is shown in the data of table 4. The general tendency was for the virulent strain to reduce acidity of the originally acid liquids in which, at the same time, it had produced its best growth. The pH of the acid liquids was changed but very little by the mild strain. On the whole, liquids with reactions around neutrality were made more alkaline by the virulent strain; comparatively, they were changed less by the mild strain. The liquids that were initially of moderate alkalinity in which the mild strain produced its maximum growth were at first made more acid by its growth and changed but little by the virulent strain. These mild cultures became slightly more alkaline than the original pH level as their age increased, while the virulent cultures displayed a greater degree of alkalinity.

TABLE 3.—*Growth[a] in Tochinai liquid medium of different strains of Fusarium bulbigenum var. lycopersici as influenced by various pH reactions of the medium*

Reaction of medium when inoculated	Weight produced by the Fusarium strains indicated										
	R5–6[b]	A15–8[c]	R5–6 and A15–8 mixed			RS 29[d]			IR 13[e]		
	Series 4 Test No.	Series 4 Test No.	Series 1 Test No.			Series 1 Test No.			Series 1 Test No.		
	1	1	1	2	3	1	2	3	1	2	3
pH	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1.7	.01	.00	.02	.01	.01	.02	.01	.00	.01	.01	.01
2.1	.03	.01	.06	.04	.05	.03	.03	.04	.03	.03	.03
2.5	.23	.04	.36	.35	.16	.09	.14	.07	.14	.06	.08
2.9	.35	.26	.33	.23	.29	.15	.14	.20	**.21**	**.22**	**.17**
3.2	.49	.....	.23	.40	.41	.22	.25	.24	**.20**	**.22**	.09
3.5	.46	.26	.45	.46	.36	.25	.20	.22	.17	.16	.17
3.8	.65	.27	.54	.46	.49	.27	**.55**	**.50**	.15	.19	.22
4.2	**.89**	.27	**.84**	**.80**	**.84**	**.40**	.31	.35	**.22**	.20	.20
4.5	**.87**	.31	**.87**	**.80**	.45	**.36**	.38	.33	.16	.22	.21
6.1	.77	**.37**	.40	**.81**	.35	**.36**	.37	**.45**	.17	.19	.22
7.1	.71	**.41**	**.73**	**.80**	**.71**	.33	.39	**.37**	.21	**.25**	**.28**
7.7	.74	**.37**	**.74**	.64	**.73**	**.41**	**.42**	**.39**	**.24**	**.24**	.23
8.4	.61	.36	**.72**	.69	**.72**	**.42**	**.44**	**.40**	**.22**	**.25**	.22
10.3	.51	.36	.56	.55	.58	.....	**.40**	.35	**.25**	**.25**	**.31**
11.4	.41	.19	.35	.38	.27	.27	.23	.29	.10	.15	.22
11.7	.30	.00	.24	.29	.25	.12	.14	.14	.12	.00	.00

[a] Growth recorded as the mean of air-dry weights of mats from 3 cultures in each treatment. Cultures incubated 16 days in the dark at approximately 27.5° C.

[b] Stable, pure-line, highly virulent strain. See table 1 and text for description.

[c] Stable, pure-line, very mild pathogenically. See table 1 and text for description.

[d] Unstable, vigorously saltating strain, severe at times but erratic in its pathogenic behavior (11).

[e] Unstable, saltating strain, of medium somewhat erratic pathogenicity (11).

CHANGE IN pH OF LIQUID CULTURES INDICATIVE OF RELATIVE PATHOGENICITY

Isolates of Known Pathogenicity

As the curves of progressive pH change by the highly virulent and very mild strains of the organism were studied, it seemed probable that comparative degrees of acidity developed in liquid cultures of isolates unknown as to disease capacity might indicate their relative pathogenicities. It can be seen from reference to the curves presented in figure 1, A, that in R5–6 and A15–8 the greatest spread between pH readings occurred during the third week of incubation. Cultures of this approximate age, therefore, were tested for acidity in these studies.

The first group of cultures used were the 28 isolates that had previously been studied (11) for relative pathogenicities correlated with culture classes. All isolates were grown on the differential medium described above under "Materials and Methods," they were checked as to appearance, and cultures 3 weeks old were used as source of inoculum for triplicate sets of flasks of liquid medium. A number of representative members in each of the 5 culture classes were used for pH tests after 9 days incubation, and all of

TABLE 4.—*Changes in reaction of culture liquids when inoculated at different initial pH levels*[a] *using the virulent R5–6, and the mildly pathogenic A15–8 strains of Fusarium bulbigenum var. lycopersici and allowed to grow for various periods*[b]

Experiment 1[c] Incubated 9 days			Experiment 2 Incubated 12 days			Experiment 3[d] Incubated 16 days			Experiment 4 Incubated 19 days		
Original reading	After growth		Original reading	After growth		Original reading	After growth		Original reading	After growth	
	R5–6	A15–8		R5–6	A15–8		R5–6	A15–8		R5–6	A15–8
pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
.....	.....	.....	.....	.....	.....	2.9	2.8	2.7	2.7	2.3	2.6
3.2	3.5	3.2	3.2	3.7	3.2	3.2	3.5	.....	3.0	4.2	3.2
3.6	4.1	3.8	.....	.....	.....	3.5	4.7	3.6	3.2	7.6	4.0
.....	.....	.....	.....	.....	.....	3.8	5.2	4.3	3.6	8.3	4.9
4.2	4.7*[e]	4.7	4.3	7.3*	4.8	4.2	6.9*	4.4	4.0	8.3*	5.6
.....	.....	.....	.....	.....	.....	4.5	7.6*	4.9	4.3	8.3*	6.4
4.7	6.7*	5.2	.....	.....	.....	.....	.....	.....	4.6	8.4*	6.6
.....	.....	.....	5.1	8.0	5.2	.....	.....	.....	5.1	8.5	6.8
6.0	7.3	5.3	6.1	8.0	5.6	6.1	7.5	6.0*	6.1	8.5	7.7*
6.7	8.0	5.6	.....	.....	.....	.....	.....	.....	6.6	8.6	7.7*
7.2	8.1	6.2	.....	.....	.....	7.1	7.5	.....*	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	7.4	8.6	7.9*
7.7	7.9	6.7*	7.8	8.1	6.1*	7.7	7.5	6.5*	.....	.....	.....
8.1	7.9	6.7*	8.2	8.1	6.3*	.....	.....	.....	8.2	8.8	7.9*
8.4	7.9	7.0	8.6	8.2	6.3*	8.4	7.6	6.9	.....	.....	.....
.....	.....	.....	8.8	7.8	6.5	.....	.....	.....	8.8	8.8	8.1
.....	.....	.....	9.0	7.4	6.8	.....	.....	.....	9.1	8.8	8.2
.....	.....	.....	.....	.....	.....	.....	.....	.....	9.6	8.8	8.2
.....	.....	.....	.....	.....	.....	.....	.....	.....	9.8	8.8	8.5

[a] Liquids of Tochinai formula, regulated to different pH points (original readings) by using dilute HCl and NaOH solutions. Data not included from extremely acid or alkaline liquids in which growth was absent or much reduced.

[b] The 9- and 16-day series incubated in the dark at approximately 27.5° C. Others at irregular room temperatures.

[c] Data from the same experiment as series 3, table 2.

[d] Data from the same experiment as series 4, table 3.

[e] Asterisks(*) denote most vigorous appearing mats in culture at end of incubation period. That such appearance is a correct estimate of the maximum mat development may be seen by comparing weights of series 3, table 2, with data in experiment 1 above, also series 4, table 3, with experiment 3 above. The actual mat weights were secured from the liquids tested for pH in experiments 1 and 3.

the strains were tested after growing 18 days (Table 5). For points of reference it should be kept in mind that No. 5 was the "parent" for the pure-line strain R5–6 used in other parts of these studies, and No. 15 was the "parent" for the pure-line strain A15–8.

It may be seen that after 9 days there was indication that the most highly pathogenic cultures were closer to neutrality than the milder strains, which

TABLE 5.—*Change in pH of liquid medium*[a] *due to growth of isolates of tomato wilt Fusarium that had been previously studied for relative pathogenicities*[b]

Isolate	Culture class	Reactions of inoculated liquids	
		9 days incubation[c]	18 days incubation[c]
No.		*pH*	*pH*
8	Raised	.....	8.2
5	"	6.2	8.3
21	"	.....	8.3
17	"	6.3	8.2
7	Raised sclerotial	.....	7.8
9	" "	.....	7.8
4	" "	6.3	7.9
12	" "	6.3	8.1
16	" "	6.2	8.1
2	" "	.....	7.8
29	" "	.....	8.1
11	Intermediate raised	6.2	7.6
26	" "	.....	7.4
13	" "	5.3	7.7
23	" "	6.2	7.2
20	" "	.....	7.3
14	" "	.....	7.3
3	Intermediate appressed	5.6	7.3
28	" "	5.4	7.2
22	" "	.....	7.2
10	" "	.....	7.2
19	" "	5.5	7.6
18	Appressed	.....	6.5
27	"	.....	6.9
6	"	4.9	6.9
1	"	5.0	6.3
24	"	5.5	6.0
15	"	4.8	5.5
	Sterile liquid	6.0	6.1

[a] Tochinai medium, formula given elsewhere (10); initial pH value, 6.0.

[b] Arranged according to disease evaluations as reported in a previous paper (11, p. 14); most virulent at top, in descending order of pathogenicity with mildest at bottom. Nos. 25 and 30 not included since they were anomalous members of the 30 isolates tested. It should be noted that later work has shown that No. 5 is slightly more pathogenic and stable than No. 8.

[c] Incubated in the dark at approximately 27.5° C.

were slightly on the acid side. When a total of 18 days had passed, differences in pH were even more notable: high virulence was accompanied by increased alkalinity; the mildest strains were most acid in reaction; and with the intermediate strains, the pH lay between the extremes.

ISOLATES OF UNKNOWN PATHOGENICITY

Stems of wilted tomatoes were collected in 5 gardens and fields at a dis-

tance from any artificially infested experimental plot. In all, 35 single-spore isolates were secured, 29 of them representing separate isolations from plants and 6 of them appressed saltants from raised sclerotial culture types. All were grown on the special differential agar medium. They were first classed according to cultural types and then used to inoculate flasks of Tochinai liquid medium. The pH readings of the liquids were made after cultures had been incubated from 14 to 16 days. Results were as follows: the 9 raised cultures varied in pH from 7.5 to 8.2 with a mean of 7.9; the 3 raised sclerotial types varied from 7.5 to 7.8 with a mean of 7.7; the intermediate raised varied from 5.9 to 7.6 with a mean of 6.8; the 4 intermediate appressed varied from 6.1 to 6.9 with a mean of 6.6; and the 12 appressed varied from 5.5 to 6.3 with a mean of 5.8.

Another series of 14 cultures of wilt *Fusarium*, unknown as to relative pathogenicities, were studied (Table 6). These consisted of monosporic

TABLE 6.—*Monosporic tomato wilt Fusarium isolates from naturally infected plants, compared with respect to: culture class,*[a] *change in pH of medium,*[b] *and pathogenic evaluation*[c]

Isolate	Culture class	Reaction of liquid	Disease index from host	
			Bonny Best	Marglobe
			Mean value[d]	Mean value[d]
No.		*pH*		
32	R	8.3	9.76 ± 0.77	6.16 ± 0.30
33	R	8.3	11.84 ± .81	6.56 ± .36
40	R	8.1	10.16 ± .86	5.68 ± .36
34	RS	7.9	7.28 ± .49	5.88 ± .63
37	RS	8.1	12.72 ± .64	5.76 ± .42
39	RS	8.0	8.04 ± .68	4.40 ± .26
35	IR	6.7	9.60 ± .66	7.16 ± .36
36	IR	7.4	5.88 ± .36	3.64 ± .02
38	IR	6.6	8.24 ± .69	4.36 ± .29
44	IA	7.1	6.04 ± .30	3.48 ± .02
43	IA	7.3	7.00 ± .30	4.08 ± .24
31	IA	6.7	6.76 ± .38	5.32 ± .38
41	A	5.9	5.80 ± .39	3.20 ± .23
42	A	5.8	5.44 ± .40	4.08 ± .01
R5–6[e]	R	8.3	13.72 ± .52	9.48 ± .54
A15–8[e]	A	5.9	0.68 ± .02	0.48 ± .02

[a] Classes defined and illustrated in other reports (11, 12).

[b] Tochinai liquid (10) used, original pH 6.1, flasks incubated 18 days in the dark at 27.5° ± 1.6° C.

[c] Data from 20 plants in each case. Standard technique (10) used to obtain disease evaluations.

[d] Includes arithmetical means and standard error.

[e] Included for reference purposes.

isolates from separate wilted plants from a naturally infested field. The isolates were grown on the differential agar medium and first grouped according to culture classes. They were then used to inoculate flasks of liquid and after 18 days the cultures were tested for pH values. Disease index (relative pathogenicity) was secured through the use of standard technique (10) on the susceptible host, Bonny Best and the tolerant Marglobe.

The isolates in the R class produced strongly alkaline pH reactions of from 8.1 to 8.3, and pathogenicity indexes were uniformly high. The RS-class isolates produced pH reactions from 7.9 to 8.1 with high but irregular pathogenicities. Such irregularities have been noted already (11, 12) as characteristic of the RS culture class. In both the IR- and IA-class groups, pH reactions were from 6.6 to 7.4, all so close to the neutral point as to suggest little variation between cultures. In these data, however, there does seem to be an indication that the IR cultures were slightly more pathogenic than the IA cultures. In the two A-class cultures pH reactions showed considerable acidity, being 5.9 and 5.8 and in these the pathogenicities were the lowest of any of the unknown cultures.

Finally, it is of special interest to compare these data with those obtained on the two known strains, R5–6, which had been particularly studied and selected for its high pathogenicity, and A15–8, studied and selected for its mild disease effects: R5–6 produced a pH of 8.3 equal to the highest produced by any R or RS culture, and it had a pathogenicity on either Bonny Best or Marglobe that was slightly higher than the comparable reaction of any other strain of the organism; A15–8 produced a pH of 5.9 that was similar to the reaction noted for other A cultures, and it had much the lowest pathogenicities on both Bonny Best and Marglobe. The other A strains had pathogenicity indexes that, while they were low when compared with other strains, were at the same time considerably greater than A15–8.

In general these data indicate that differences are unquestionable between R and A cultures as regards appearance, changes produced in pH, and pathogenicities; the two intermediate types (IR and IA), as their names and cultural characters signify, are definitely between the extremes exhibited by the organisms that develop high pH and pathogenicity and low pH and pathogenicity; the intermediate types would appear to be significantly different from the A group on the one hand and either the R or RS group on the other.

DISCUSSION

Where certain phases of the work just presented have necessarily paralleled the investigations of others, *i.e.*, Sherwood (9), Scott (7), White (13), Pritham and Anderson (5), and Luz (3), comparisons of results show that they have not differed greatly beyond what might be expected, considering the variations in conditions and materials represented.

The general shapes of the curves depicting change in mat weight and correlative variation in pH of culture medium over a period of time (Fig. 1, A and B) is in general agreement with similar findings reported by Luz (3). His curves were less regular than these, which indicates the probability that he used an isolate more unstable in growth character than either strain A15–8 or R5–6. Luz also noted the same reduction in pH of medium occurring during the first few days of growth as are indicated in figure 1, A. Pritham and Anderson (5) reported that they did not see such a change. In the present case this reduction in pH was observed a number of times

and was always readily determined if the tests were made early enough in the incubation period. Neither Luz nor Pritham and Anderson grew cultures that had end reactions (about pH 7.5 and 7.3) that were quite as high as are indicated in figure 1, A (pH 8.7). This is not to be wondered at, however, for, in neither case, did the other experimenters work under the same conditions or with exactly the same materials as those of the present studies.

Several workers have found that the tomato *Fusarium* produced maximum amounts of growth at two separate points of H-ion concentration, with a considerably smaller amount between these points. Such results have led to the term "bimodal phenomenon" in this connection. This was believed by Scott (8) to be attributable to the isoelectric point of the fungus mycelium, which he found was about pH 5.4. There has been considerable divergence in results among workers with regard to the pH value at which the lowered point between two maxima of growth occurred: Sherwood (9) noted in one experiment that the maxima were on either side of a minimum of pH 7.8 to 8.2; in another experiment this minimum came at 6.6 to 7.0; Scott (7) concluded after a number of experiments that the organism produced one maximum at about pH 4.5 to 5.3, a much reduced growth at 5.3 to 5.8, and a second maximum at about 5.9 to 6.9; White (13) said "all strains showed a double maximum," the first maximum varying with strains, but most generally at 4.0 to 5.5 "and was followed by a minimum at pH 5 to 7, with the second maximum at a point above pH 7"; Pritham and Anderson (5) presented a curve that showed two maximum points with between them a reduction of growth at a little above pH 7.0.

In the results here presented (Fig. 1, and Tables 2, 3 and 4), no "double maximum" was demonstrable, if relatively stable strains (A15–8 and R5–6) were studied separately in culture series. When, however, these were mixed and used as inoculum, striking irregularities in mat weights developed, which might well be interpreted as of "bimodal" nature, and perhaps even typical of the organism, were it not known that the inoculum consisted of a mixture of strains differing in cultural characteristics. In addition, data from a number of other cultures from unstable strains resulted likewise in marked irregularities, due apparently to saltations, that would fit well into the bimodality concept. Reductions in growth, so-called minimum points between maxima, were found in the present studies at around the following pH reactions: 4.2, 4.5, 4.5, 5.5, 6.1, 6.1, 6.1, 7.1, 7.7, 7.7, and 7.7 to 8.4, and such variation compares well with the differing reports of others mentioned above. Reports of double maxima may signify that the experimenter has used a culture influenced by saltant action, which would in effect have been, therefore, a mixed culture. It also appears that the point of reduced growth between two maxima does not necessarily occur at the organism's isoelectric point defined by Scott (8).

White (13) experimented with pH effects on growth of 24 tomato *Fusarium* strains, and attempted to correlate cultural with pathogenic differences. His work is not strictly comparable to that here presented,

since he described the characteristics of his cultures on agar slants in test tubes 14 months after inoculation. It has been shown (11, 12) that only by continual vigilance, with cultures in Petri dishes, and by repeated reselection at fairly short intervals, is it possible to be certain of maintaining continuity of growth class in the cultural history of isolates. Recently refinements have been developed (10) in the technique used for testing relative pathogenicities of tomato *Fusarium* strains. White's one experiment along this line was not conclusive, as he himself indicated, and he found it difficult to correlate results of cultural studies with pathogenicity findings. In view of this and the later findings of Haymaker (2), and White's own descriptions, it is fairly probable that many if not all the strains White worked with were actively varying, although this condition may not have been recognized.

The studies herein reported add another method of indicating variation among strains of the tomato wilt *Fusarium*. Along with other growth reactions, isolates may be grown in Tochinai liquid at about 27.5° C. for a little more than 2 weeks, and then tested for pH. Judging from these findings on pH reactions, the highest will be produced by the most virulent cultures, the lowest by the mildest cultures, and those of intermediate virulence will show intermediate pH values. In such tests it is necessary to use standard, known, highly virulent, very mild, and intermediately pathogenic strains along with the unknown cultures for comparison and evaluation of the data obtained.

The investigator might well expect that a dark-colored, appressed, and slimy culture from one source would be very different in physiological behavior from a white, woolly culture from another source. Such difference has been demonstrated in the case of varying cultures, both from numerous separate sources and from only one source. It is of special interest, moreover, when it is recalled that appressed types are readily obtained from originally raised cultures, and that significantly higher raised types may be secured from appressed cultures (12).

From data given in the present paper it is far from possible to know what is the basis for the higher virulence of *Fusarium* cultures of the raised type in comparison with the mildly pathogenic appressed type. Any conclusions as to whether virulence is correlated with either inhibition or more vigorous growth in culture must await further study. At present, it would depend upon the criterion selected for comparison. For example: in liquids the virulent R5–6 strain produced much greater mat weight than the mild strain A15–8. The heavier growth by the virulent organism was quickly followed, however, by rapid loss in weight, probably because of autolysis. The mild strain, after reaching its greatest mat weight, was comparatively less affected by losses resulting from autolysis. On the other hand, on agar surfaces the mild strain covered significantly larger areas than did the virulent one.

SUMMARY

Strains of the tomato-wilt *Fusarium* that differ in pathogenicity are also different in their physiology, as indicated in certain pH relationships and relative amounts of growth.

In liquid culture a highly virulent strain produced its maximum weight in 12 days, while a mild strain required nearly twice this period. The former produced the greater weight in liquid, but, on agar, the mild strain covered the greater area.

In liquid media the maximum growth of the virulent strain was produced only at a pH of around 4.0, and of the mild strain only around 8.0. On agars both had approximately equal maximum points at slightly above neutral. In series of liquid media having a wide range of pH reactions, cultures inoculated with mixed mild and virulent strains produced maxima at two points, as did cultures inoculated with actively saltating, unstable strains. The virulent and mild strains changed the original pH reactions in different degree.

When the culture liquids of a large number of isolates were tested for pH reactions and the pathogenicities of the organisms were determined, it was evident that under parallel conditions, virulent strains produced most alkalinity, mild ones most acidity, and those of intermediate pathogenicity developed reactions between the extremes.

This paper also reports a new differential agar medium by which it was possible to separate with greater ease the variant culture types of the tomato-wilt *Fusarium* strains used in these studies.

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THE EFFECT OF TOBACCO-MOSAIC VIRUS ON CELLULAR RESPIRATION[1]

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INTRODUCTION

Viruses apparently do not respire when removed from contact with living suscept cells (1, 5). Whether viruses exhibit respiratory phenomena during their multiplication in living cells is not known. There is evidence, however, that multiplication of tobacco-mosaic virus protein (*Marmor tabaci* Holmes) may be dependent on cyanide-sensitive respiratory catalysts of the cell (21).

Viruses may represent the extreme in parasitic adaptation of organisms that have become dependent upon their hosts for essentially all metabolic functions, including respiration. On the other hand, it is also possible that viruses represent more or less altered proteins of "host" origin that are capable of some form of multiplication in various "susceptible" species. The source of energy for synthesis of virus proteins is an important consideration regardless of the view concerning their ultimate origin. One approach to this problem is through a study of the relation of the virus to the specific respiratory systems of the cell. This paper presents data from such investigations.

From the available literature it is not possible to determine whether the action of the virus on the respiratory systems consists of a primary chemical action or whether it is due to secondary morphological or physiological changes in the affected organ.

Comparisons of respiratory activity in healthy and diseased tissues that involve samples from different individuals, or different leaves from the same plant, may lead to erroneous conclusions in this respect because of the wide variability in respiratory characteristics that often occurs in such cases. In the present study by use of half-leaf comparison techniques, variations due to genetic, morphological, or physiological differences between control and experimental tissues were practically eliminated. Approximately equal numbers of cells in the diseased and control samples were assured by the use of leaves that had either ceased or almost ceased growing. With these techniques it has been possible to demonstrate that tobacco-mosaic virus affects respiration both quantitatively and qualitatively, influencing the activity of particular respiratory components in a definite time sequence following inoculation. These changes have been followed in single cells as well as in larger masses of tissue.

MATERIALS AND METHODS

A single lesion strain of strongly mottling *Marmor tabaci* was used in all

[1] Scientific Paper A1. Contribution No. 1717 of the Maryland Agricultural Experiment Station (Department of Botany).

experiments, unless otherwise noted. In some experiments a "yellow" mutant derived from the parent "green" strain was used. Inoculations were made by rubbing halves of fully expanded *Nicotiana tabacum* Turkish leaves with a cheesecloth-covered spatula dipped in freshly extracted infective juice. The opposite half of the same leaf was similarly rubbed with a moistened sterile spatula to simulate the wounding injury of inoculation. Vigorous pot- or bench-grown plants were used in all experiments. Inoculations were made when the plants were 12 to 24 inches high but before development of flower buds.

Leaf samples with an area of 1 sq. cm. were removed for respirometer measurements with a steel die. Only tissue lying between the secondary veins was used. Measurements of protoplasmic streaming were made in marginal epidermal cells of the leaf blade. With these techniques it was possible to measure oxygen uptake and protoplasmic streaming in the same leaf. One to 3 mm. of the mesophyll was always included in the sample to insulate the marginal epidermal cells from the injury attendant to cutting out the sample. These marginal cells are ideally suited to measurements of streaming, as they can readily be viewed under the oil-immersion lens, and present a large surface to the irrigating fluid from which they derive their oxygen supply. In measuring cyclotic rate it is extremely important to accurately control the rate of solution flow and to keep the tissue under generally uniform conditions. The cells under observation must be held firmly in place, be freely bathed with the irrigating solution, and not be subject to mechanical injury. The apparatus developed by Aston[2] (unpublished) was found ideally suited to this work and was used in all experiments reported here. Single cells were often kept under observation and in an active state of cyclosis for as long as 4 days.

Measurements of virus concentration in experimental samples were made either immediately following their removal from the respirometers or the samples were stored at 3° C. for several hours before testing. The respirometer samples, after measurement of respiration, were ground up in water, then diluted (about 120 mg. fresh tissue per 3 cc. of water) and the extract rubbed onto half-leaves of *Nicotiana glutinosa* or hybrids of it. The opposite halves of the same leaves were rubbed either with a similarly prepared extract from the control tissue or compared with a standard virus preparation (a similar dilution made from fully invaded Turkish tobacco leaves). The number of necrotic lesions developed on the leaves of these hybrids was used as a measure of virus concentration in the samples.

Oxygen uptake was measured in a polarographic microrespirometer, the tissue being immersed in a ground solution, as previously described by one of us (8) or in air in a modified Fenn (12) differential volumeter (each vessel 7.5 cc. capacity). In the former, significant determinations of oxygen-uptake could be made every 3 minutes on approximately 20 mg. of green leaf

[2] The authors wish to express their appreciation of valuable assistance rendered by Mr. Arthur Aston in design and construction of apparatus used in measuring protoplasmic streaming.

tissue (1 sq. cm.). Five to 7 similar samples were required to give significant measurements every 3 minutes in the Fenn respirometer. The formula for pressure and temperature correction, as given by Fenn (12, pp. 7–8), was used in computing the actual oxygen uptake. Control and infected tissues were run simultaneously, i.e., allowed to "pull against each other." In application of the S.T.P. correction formula the slight error (less than 1 per cent) resulting from reduction in pressure in the comparison volumeter due to respired oxygen was ignored. Oxygen-uptake measurements were made with the tissue in complete darkness to prevent evolution of oxygen in photosynthesis. Rates of oxygen consumption are expressed in *micro liters per hour.*

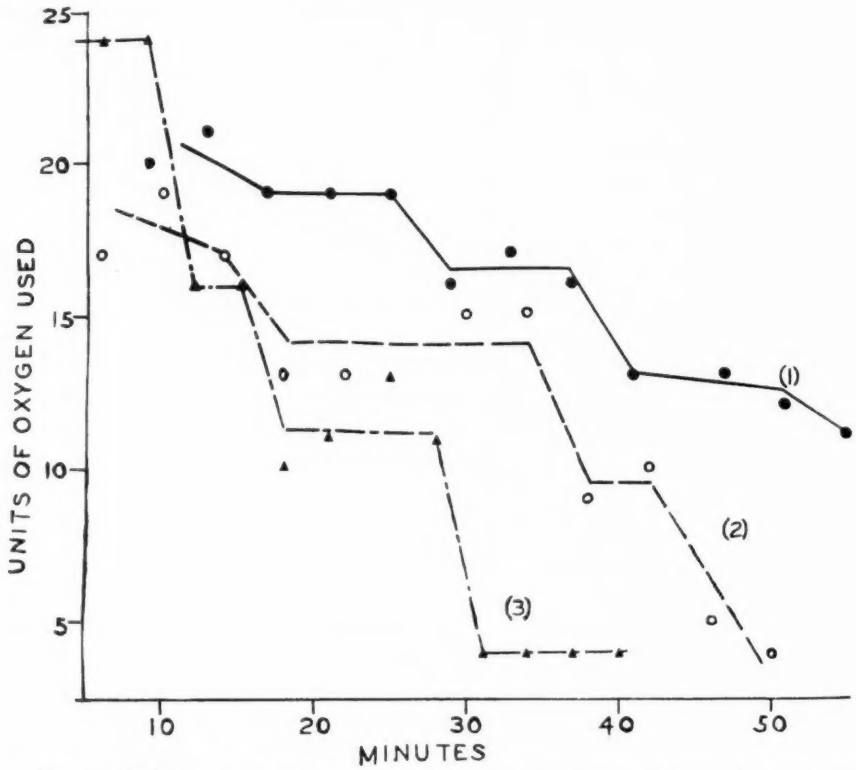


FIG. 1. Relative rates of oxygen-uptake by healthy tissues of three different full-grown Turkish tobacco leaves as measured in the polarographic microrespirometer.

EXPERIMENTAL

Respiration Systems in the Healthy Cell

Measurements in the Polarographic Respirometer. If measurements are made in the polarographic respirometer of oxygen uptake by healthy tissues of full-grown Turkish tobacco leaves, a compound curve is obtained. The results of 3 experiments are illustrated in figure 1. These curves represent the rates of oxygen uptake during progressive suffocation. It is evident that the rate of oxygen uptake by the tissue does not diminish in direct

proportion to the lowering of oxygen tension in the surrounding solution. Respiration first proceeds at maximum velocity at the highest tensions of oxygen (first part of the time period), then suddenly (within 2 to 4 minutes) shifts to an intermediate rate, which carries on until one or two more inflections, which result in the lowest rates of oxygen uptake. Further suffocation results in a gradual reduction in oxygen respiration, which, if not relieved by aeration, finally results in death of the tissues. Treatment of leaves with KCN 0.008 molar, which also inhibited the peroxidase and catalase activity of the ground-up tissues as indicated by guiacum and hydrogen peroxide, respectively, reduces the rate of oxygen uptake of the living cells. Polarographic measurements have shown that HCN inhibits those components of oxygen respiration responsible for the high velocities of uptake that occur at the higher oxygen tensions during progressive suffocation (Fig.

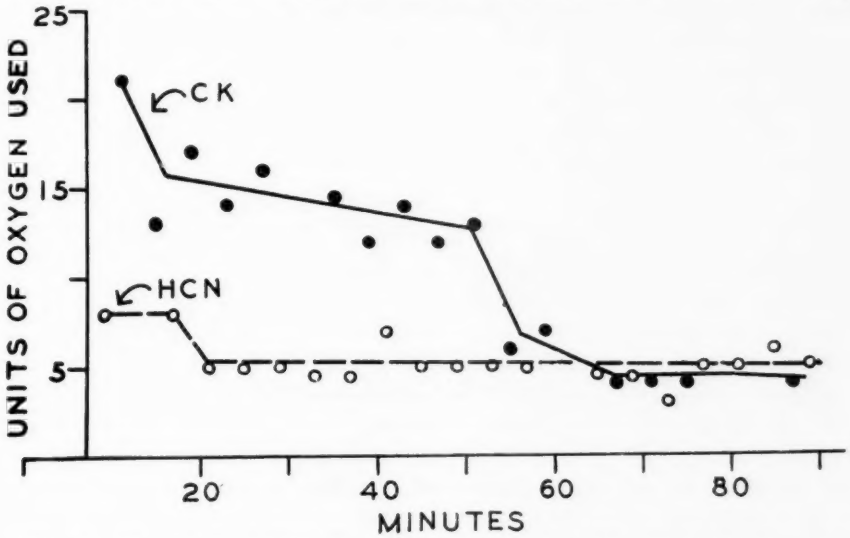


FIG. 2. The effect of HCN on the respiration of healthy Turkish tobacco leaf tissues. Both samples were from the same leaf, oxygen-respiration being measured in the polarographic microrespirometer.

2). This is in accord with previous work on *Avena* coleoptiles (8, 14). Analysis of a large number of experiments indicated that at least 3 respiration components by their combined action enable maximum oxygen uptake. Three-step reductions in respiration rate during progressive suffocation were often obtained. Tissue from some leaves, however, failed to give clear-cut 3-step reductions or showed more than 2 inflection points (Fig. 1, curve 3). Measurement of such tissues in solutions saturated with pure oxygen revealed that diffusion of oxygen into the tissues was a limiting factor, for the increase in oxygen tension resulted in a marked increase in the maximal respiration rate. With the polarographic method some limitation in oxygen diffusion to the interior of the tobacco leaf probably always occurs. Such limitations make very difficult any accurate comparisons between healthy

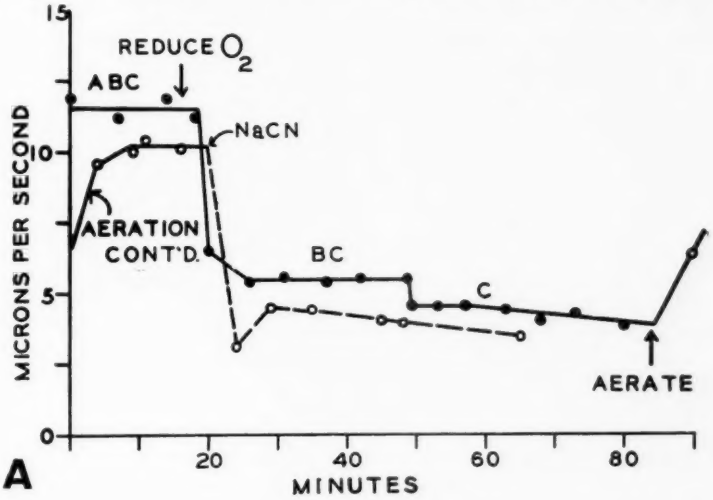
and diseased samples at maximal oxygen consumption in the polarograph. This difficulty is accentuated because of *compensatory* changes that develop in the respiratory catalytic systems that show a differential response to oxygen tensions. As will be pointed out below the multiplication of the virus results in a reduction in the activity of one of the cyanide-sensitive components of respiration that requires a high oxygen tension in order to function, and is followed by a marked increase in a cyanide-resistant component that is able to work at relatively low oxygen tensions. Thus, during progressive suffocation in the polarographic respirometer, the *outer* tissue layers of a diseased sample may exhibit a lower respiration rate than corresponding cells of the healthy leaf, whereas the *interior* cells of the infected sample may respire more rapidly than the corresponding healthy cells. This latter behavior is due to the increased activity in the diseased leaf of the particular respiration component able to function at low tensions of oxygen.

In spite of these limitations the polarographic method proved to be of definite value because the results obtained indicated the existence of different respiration systems in tobacco separable by the techniques of progressive suffocation and enzyme poisoning. It is possible to interpret all of the polarographic data in the light of results obtained from measurements of protoplasmic streaming and of oxygen uptake in the Fenn respirometer.

Measurements of Protoplasmic Streaming. It has been previously determined that protoplasmic streaming in tobacco leaf cells is oxygen-sensitive and responds to cyanide in about the same way that oxygen uptake does (22). A further study was made in the hope that rate of streaming (cyclosis) might be used to more precisely determine the above-mentioned different respiratory components in individual cells. Experiments have shown that the response of tobacco cells to suffocation (diminishing oxygen supply) and cyanide is essentially the same in both cyclosis and oxygen respiration. Three different oxidation components contributing to the rate of protoplasmic streaming have been determined by progressive suffocation (Fig. 3, A and B). The first of these, the "A component" ceases to function at a certain oxygen level as measured by cyclosis, later a "B component" drops out, whereas at the lowest oxygen tensions protoplasmic streaming is energized only by a "C component." The A and B components also can be reversibly inhibited with NaCN or KCN (Fig. 3, A and B). Potassium cyanide, 0.0002 molar, is as effective as 0.002 molar in reducing the rate of streaming. The relationships between streaming and respiration, apparent from a comparison of figures 1 and 3, are particularly striking when mosaic-diseased and healthy cells are compared (Tables 1 and 2). The action of the virus results in the same characteristic changes in both. Repeated experiments have shown that the rate of protoplasmic streaming can be used as an index to respiratory activity in single cells.

Measurements in the Fenn Respirometer. Measurements of oxygen uptake by healthy leaf tissues in the Fenn respirometer gave results that, in general, accord with those obtained by the polarograph or by protoplasmic

streaming determinations. With the Fenn respirometer volumes employed (approximately 7.5 cc.) it was not possible to separate the A, B, and C systems by auto-suffocation. However, respiration inhibitors can be used to advantage. HCN inhibited from 40 to 50 per cent of the total respiration



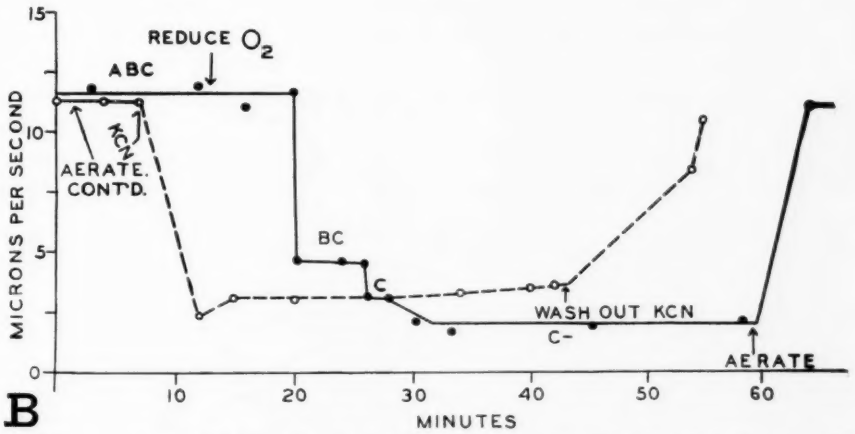


FIG. 3. A. Cyclotic analysis of a healthy epidermal cell of Turkish tobacco. The rates of protoplasmic streaming are recorded in microns per second. Solid line indicates rate during progressive suffocation and the dotted line behavior following treatment with NaCN. The rates of protoplasmic streaming due to the different respiration systems (A, B and C) are indicated on the curve. B. Similar type of analysis of another healthy cell under conditions of more rapid suffocation, and using KCN as the inhibitor.

providing certain conditions were realized (Fig. 5). Preliminary investigation demonstrated that *cyanide-sensitive respiration in the interior of the leaf is inhibited in varying degrees unless the stomata in both upper and lower epidermes are kept open*, and excessive mechanical or other injury

TABLE 1.—*Activities of oxidation components A, B and C in 8 pairs (healthy-infected) of epidermal cells of Nicotiana tabacum at various periods following inoculation with tobacco mosaic virus (Marmor tabaci). Analyses based on measurements of inflection points during progressive suffocation. Each pair of cells represents typical cells from opposite sides of the same leaf*

Expt. No.	Leaf half in which cell was measured	Hours after inoculation	Relative amount hexagonal crystalline material	Activity of oxidation components A, B and C (rate of cyclosis in microns per second)					Temperature in °C.
				ABC	BC	A	B	C	
1	CK.	.....	.....	10.9	5.6	5.3	1.5	4.1	27.5
	INOC.	77	*[a]	6.5	5.6	0.9	1.8	3.8	
2	CK.	.....	.....	13.4	6.9	6.5	1.8	5.1	27.5
	INOC.	80	abnormal vacuolization	12.9	7.6	5.3	2.5	5.1	
3	CK.	.....	.....	12.6	8.1	4.5	1.9	6.2	28.0
	INOC.	84	*	.....	9.3	0	1.5	7.8	
4	CK.	.....	.....	11.5	6.5	5.0	2.3	4.2	27.5
	INOC.	132	**	.....	11.3	0	2.2	9.1	
5	CK.	.....	.....	11.3	7.0	4.3	1.4	5.6	27.5
	INOC.	202	*	.....	9.0	0	1.8	7.2	
6	CK.	.....	.....	13.2	6.5	6.7	2.0	4.5	27.5
	INOC.	215	***	.....	8.0	0	1.8	6.2	
7	CK.	.....	.....	15.0	7.0	8.0	1.7	5.3	28.0
	INOC.	218	***	.....	10.4	0	2.1	8.3	
8	CK.	.....	.....	13.2	6.1	7.1	1.7	4.4	27.5
	INOC.	293	***	.....	10.7	0	3.1	7.6	

[a] * = small amount of hexagonal crystalline material; ** = twice as much; *** = three times as much.

TABLE 2.—*Activity of cyanide-insensitive oxygen respiration in leaves of N. tabacum after inoculation with tobacco mosaic virus (all measurements made in Fenn respirometer)*

No. of leaf	Hours after inoculation	Micro liters O_2 consumed per hour (samples approx. 120 mg.)		Percent. increase in O_2 uptake in inoculated half	Test for virus in expt. samples (uninoc./inoc.)[b]	Temp. during test in °C.
		Uninoculated half	Inoculated half			
1.	not inocul.	120.8 (left)	126.0 (right)	dif. = 4.3	.....	27.5
2.	Do	40.6 (left)	40.8 (right)	dif. = 0.5	.....	27.4
3.	30	53.4	51.5	− 5.5	0/39[c]	27.5
4.[a]	51	62.0	60.6	− 2.3	0/105[c]	27.7
5.	92	50.2	50.2	0.0	31/634[c]	27.6
6.	116	62.0	81.6	24.1	5/670[c]	27.6
7.	123	56.8	67.0	15.3	0/844[c]	27.7
8.	168	56.9	80.6	29.5	0/648	27.5
9.	198	92.1	117.7	21.8	9/778	27.8
10.	216	25.6	46.6	45.1	0/1915	27.5
11.	218	41.5	49.2	15.7	3/1043	27.7
12.	237	15.3	30.8	50.4	0/264	28.4
13.	291	66.5	76.8	13.5	11/649	27.8

[a] This sample run with closed stomata only, no HCN was used.

[b] Number of local lesions developed on half leaves of *N. glutinosa* hybrid.

[c] Uninoculated and inoculated samples tested on different plants of the *N. glutinosa* hybrid.

TABLE 3.—*Rates of cyclosis in epidermal cells extending from the yellow margin of a primary lesion of yellow mosaic into the normal-appearing area around the periphery. Cell position number one was nearest to the center of the lesion*

Position	Mosaic-diseased cells Rate of cyclosis in microns per second		Healthy cells Rate of cyclosis in microns per second	
	Before KCN	In KCN[a]	Before KCN	After KCN[a]
1	10.7	11.1	11.6	4.4
2	10.2	11.4	11.3	4.3
3	11.1	8.7	10.7	4.1
4	8.4	7.3	9.5	4.0
5	8.4	6.5	8.9	3.9
6	8.1	5.2	.....	.....
7	Not measured	6.3	.....	.....

[a] Cells 1 and 2 are not the same cells as 1 and 2 in the first column, although they were in the same relative position with respect to the lesion. Cyanide actually caused a decrease in cyclosis, although much less inhibition occurred in the diseased cells.

avoided. When leaf samples were removed about 6 p.m. and floated on water in covered Petri dishes, about 40 cm. from a 100 watt Mazda lamp, the stomata were wide open by the next morning. Samples so treated could be held in the dark for periods of $\frac{1}{2}$ to 2 hours without the stomata closing. Leaves treated in this way were the only ones that gave both maximal respiration and response to cyanide.

As a result of studying oxygen respiration with the 3 techniques described above it can be concluded that oxygen uptake in healthy Turkish tobacco leaves proceeds through the combined action of at least three systems. The first of these, the "A system" is very labile, being readily blocked by cyanide or by reduction in oxygen tension. The "B system"

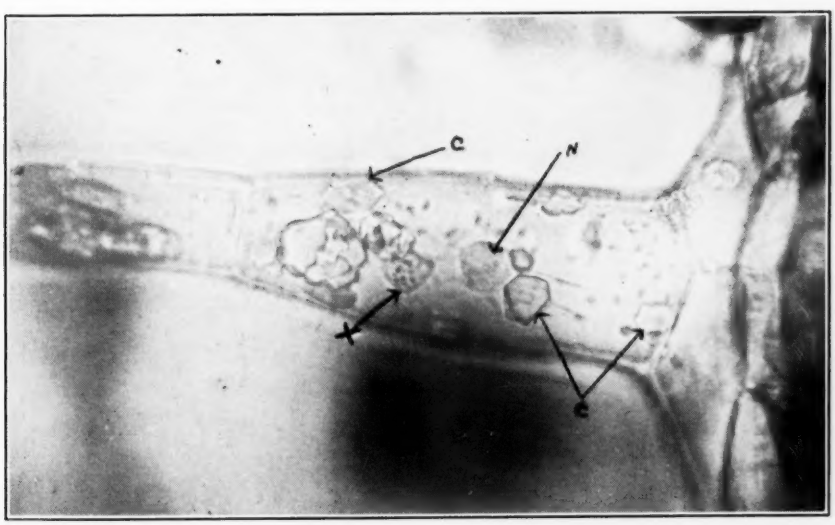


FIG. 4. Living trichome from leaf margin of *Nicotiana tabacum* Turkish infected with the "green" strain of *Marmor tabaci* Holmes. Hexagonal protein crystals, C; nucleus, N; intracellular body ("X body"), X. Approx. ×2,000.

is less labile, but can be blocked by cyanide or further reduction in oxygen tension. The third, or "C system" is the most resistant to suffocation and is not directly blocked by cyanide. Furthermore, in young leaves, the cyanide-sensitive systems are more active than in older leaves, sometimes constituting over 80 per cent of the total respiration.

The Effect of Tobacco Mosaic Virus on the A, B, and C Systems

The first respiratory symptom of infection with *Marmor tabaci* is a reduction in activity of the A system (Table 1). This effect can be detected

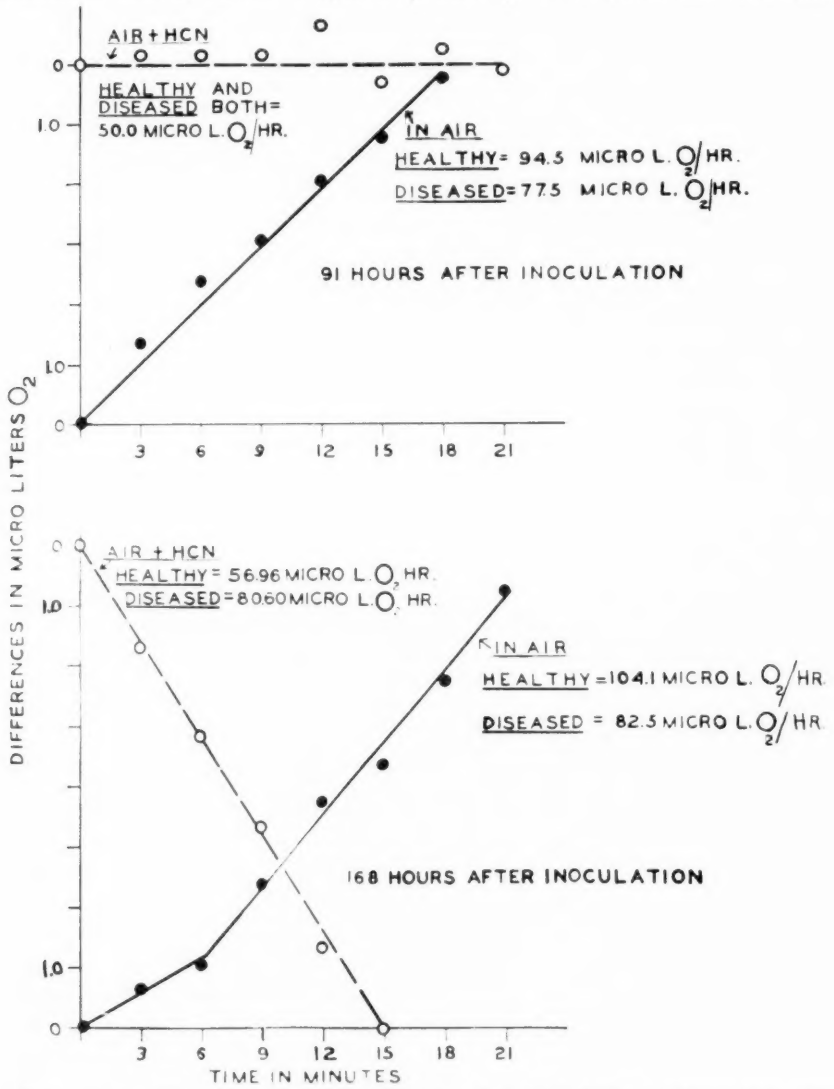

AIR + HCN
HEALTHY AND DISEASED BOTH= 50.0 MICRO L. O2/HR.
IN AIR
HEALTHY= 94.5 MICRO L. O2/HR.
DISEASED=77.5 MICRO L. O2/HR.
91 HOURS AFTER INOCULATION
DIFFERENCES IN MICRO LITERS O2
AIR + HCN
HEALTHY = 56.96 MICRO L. O2 HR.
DISEASED=80.60 MICRO L. O2 HR.
IN AIR
HEALTHY =104.1 MICRO L. O2/HR.
DISEASED = 82.5 MICRO L. O2/HR.
168 HOURS AFTER INOCULATION
TIME IN MINUTES

FIG. 5. Oxygen uptake in mosaic-diseased and healthy Turkish tobacco leaf tissue, 91 and 168 hours after inoculation. Experimental samples each consisted of 6 square centimeters (approximately 120 mg.) of tissue. Differences in oxygen uptake between the diseased and healthy samples are plotted. The absolute rate of oxygen uptake in micro liters per hour is indicated in the figure.

by cyclotic measurements in individual cells 72 hours, or less, after inoculation. It becomes manifest just before or shortly after the development of the first intracellular hexagonal protein crystals. These crystals are positive evidence of cellular infection by the virus (Fig. 4). For a detailed description of the cytological changes following mosaic infection, see Dufrenoy and Dufrenoy (10). The C system is not changed in activity until the A component is inactivated. Measurements in the Fenn respirometer have not given positive evidence of reduction in activity of the A system before 91 hours after inoculation (Fig. 5). Figure 5 shows a 53 per cent reduction in oxygen uptake in the presence of cyanide 91 hours after inoculation without apparent change in the cyanide-resistant system. Mea-

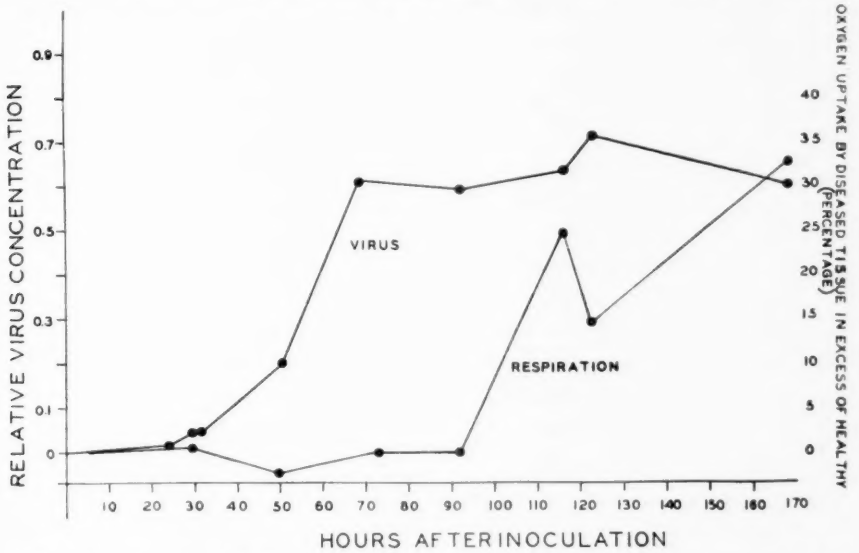


FIG. 6. The effect of *Marmor tabaci* Holmes on cyanide-resistant oxygen respiration in *Nicotiana tabacum* Turkish. The relative virus concentration has been plotted against the cyanide-resistant respiration in the same samples. Respiration is expressed as oxygen uptake by the diseased tissue in excess of the healthy tissue of the same leaf.

surements made with the cyclosis technic in a number of cells from leaf margins 48 to 72 hours after inoculation indicate that the reduction of respiration in individual cells may occur within 48 hours or less. It is believed, however, that inactivation of the A system occurs only after development of large quantities of virus. This is borne out by the relationship exhibited to formation of the hexagonal crystals, which probably indicate a high virus titre (2).

The activity of the B and C systems following infection are illustrated in table 1. It can readily be seen that 84 hours after inoculation there is a large increase in the activity of the C system. Apparently, there is little or no significant change in the activity of the B system. The relation between the approximate curve of virus multiplication and increase in activity of the C system as measured with the Fenn volumeter is shown in figure 6. It is evident that the change in the C system does not occur until after virus

multiplication has practically stopped. The increases in oxygen uptake by the C system are easily demonstrated in the Fenn respirometer by making determinations in the presence of HCN (Table 2, Fig. 6). These increases in oxygen uptake by the C system parallel increases in activity of the C system as measured by protoplasmic streaming (Table 1).

The progressive increase in activity of the C system can also be demonstrated by measuring, cell by cell, the rate of protoplasmic streaming from the center of a lesion to the normal appearing peripheral areas (Table 3). In this case the epidermal cells over the yellow lesioned area were extremely resistant to cyanide, whereas the more recently infected cells, further from the periphery of the visibly affected area, behaved more like normal cells.

In another test involving the "yellow strain" of the virus, the average rate of cyclosis in 5 infected cells was 8.5 μ per second. Following treatment with KCN the average rate was 5.7 μ. In 5 healthy cells of the same leaf the respective values were 10.8 and 3.8 μ per second. In a third test, 197 hours after inoculation, 5 infected cells exhibited an average cyclotic rate of 9.2 μ per second and 5.4 μ per second following treatment with cyanide. Corresponding data for 5 healthy cells of the same leaf were 10.7 μ per second and 2.4 μ per second, respectively. The general effects of the "yellow strain" on cellular respiration are similar to those produced by the parent "green" virus.

The first macroscopic symptoms of infection (chlorophyll destruction) with either the "green" or "yellow" strain of the virus usually appeared in the inoculated leaves 48 to 96 hours after inoculation. The "green strain" of *Marmor tabaci* produced very faint yellowish-green primary lesions in inoculated leaves, whereas the "yellow strain" produced very definite yellow local lesions. An increase in C system activity does not appear to be a necessary concomitant of chlorophyll destruction, because the latter is sometimes apparent before any change in the C system activity can be observed. In the early stages of infection with either virus cyclosis tends to become jerky and irregular, later stages concomitant with increases in C system activity are characterized by remarkable regularity and smoothness of protoplasmic streaming, together with the disappearance of larger cytoplasmic granules.

In some leaves cyclosis in both healthy and diseased cells shows a secondary poisoning effect with cyanide that is entirely distinct from ordinary reversible partial inhibition of protoplasmic streaming. When present this auto-inhibiting effect in the presence of cyanide is much more marked in diseased than in healthy cells. In these peculiar cases treatment with cyanide for 30 to 60 minutes results in accumulation of some substance or substances highly toxic to the C system. This phenomenon, which is of only occasional occurrence, will be the subject of a later paper.

DISCUSSION

Aerobic respiration of tobacco leaves involves both cyanide-sensitive and cyanide-resistant systems of catalysts. Similar systems have been demon-

strated for the respiration of other phanerograms (3, 5, 6, 8, 13). While it has not been proved, cyanide-sensitive respiration of tobacco leaves (A and B components) probably is attributable at least in part, to the activity of a cytochrome oxidase-cytochrome system. At present it is not possible to state the fundamental difference between the A and the B components, although they can be separated by varying the oxygen tension. During respiration at low oxygen tensions, or in the presence of cyanide, molecular oxygen may enter the process through the re-oxidation of the alloxazine nucleotide which is a cyanide insensitive respiratory enzyme (15, 16, 17). Thus our C system would correspond to the complex of dehydrogenations and hydrogen transfers extending from the substrate to the final donation of hydrogen by the reduced alloxazine nucleotide to oxygen. In the absence of cyanide, or, at an adequate oxygen tension, the cytochrome system with the corresponding dehydrogenases would bring about the re-oxidation of the alloxazine nucleotide, and oxygen would be "activated" by cytochrome oxidase, thus accelerating the acceptance of hydrogen. Under these conditions a more rapid uptake of oxygen would occur and the greater supply of energy would accelerate protoplasmic streaming proportionally (9). While this may represent a partial definition of the cyanide-sensitive and cyanide-resistant respiration systems in tobacco, experimental data on the exact chemical nature of higher plant respiration are too scant to warrant detailed conclusions. It is important, however, that certain respiratory systems in tobacco can be differentiated by the techniques of progressive suffocation or cyanide treatment. DuBuy and Olson (8) have described similar systems in *Avena* coleoptile using the polarographic respirometer and measurement of protoplasmic streaming. Marsh and Goddard (13) found, in mature leaves of carrot, only cyanide-resistant respiration and both cyanide-resistant and cyanide-sensitive respiration in young leaves. In *Avena* (8), however, the cyanide-sensitive system is always active, although it is greatly reduced in older plants. A similar situation occurs in tobacco.

It has been variously reported that viruses stimulate, inhibit, or have no effect on the respiration of plants susceptible to them. Cordingley, *et al.* (7) have observed that mosaic-infected tobacco leaves resemble *old* healthy leaves in their carbohydrate and protein metabolism. These authors suggested a possible lower respiration rate in mosaic-diseased leaves from their data for loss of carbohydrate.

Caldwell (4) reported increased CO_2 output by leaves of tomato infected with aucuba mosaic. This was true in normal atmosphere or in oxygen. Caldwell and Meiklejohn, using thin slices of tomato stems in Barcroft respirometers (5, 6), found that inhibition in respiration produced by M/300 cyanide was of the same order in aucuba-mosaic-diseased and healthy tissues. However, it is suggested (5, p. 485) that ". . . the enzymes concerned in the preliminary stages of respiration were more active in virus-diseased than in healthy plants." Dunlap (11) observed that leaves of young tobacco plants infected with mosaic evolved more CO_2 than similar

healthy leaves. The reverse situation held in mature leaves. Dunlap worked with field-grown plants naturally infected with mosaic. Dufrenoy and Dufrenoy (10), in a physiological and cytological study of the tobacco-mosaic disease, found greater oxygen uptake in infected cells of old tobacco leaves than in apparently healthy cells; whereas, in bud tissues, there was a reduction in oxygen uptake of over 30 per cent following infection. These results can be harmonized with the data obtained in the present investigation on the assumption that in infected buds the virus brings on an inhibition of cyanide-sensitive respiration ("A system") without at first causing an elevation in the cyanide-resistant respiration ("C system"). The increase in respiration occurring in old leaves could be accounted for on the basis of secondary increases in the relatively stable cyanide-resistant "C system."

It is not possible to explain the variance between some of these reports and the present investigation, although differences in methods of sampling or in other techniques may be involved. The use of the half-leaf technique in conjunction with different micro methods for determining respiratory activity has eliminated many variables that in certain cases make interpretation difficult. In fact, the protoplasmic-streaming technique allows measurement of respiratory activity in *single* cells. Moreover, it is a convenient and rapid method of assaying respiratory conditions in a tissue.

The primary respiratory symptom of infection with tobacco-mosaic virus is one of inhibition. A secondary symptom is a stimulation of the cyanide-resistant portion of the respiratory mechanism. If in other investigations the metabolism of a healthy plant were compared with that of diseased plants in late stages of infection, and if no special precautions were taken to insure open stomata, it is possible that the oxygen tensions within the tissues were inadequate to allow full activity of all respiratory systems. Thus the sum total of respiration of the healthy tissue could consist of a small part of the A system activity plus the B and C components. In the diseased tissues it would consist of the activity of the B system plus that of an increased C system. This would give an apparent increase in respiration in the diseased tissues. While the virus affects the respiration systems of the suscept cell, these systems in their turn apparently affect the multiplication of the virus, as shown by our data. This multiplication apparently depends upon a cyanide-poisonable component of the cell (21). Recent work with gaseous cyanide (Woods, unpublished) has further demonstrated the specific and *reversible* nature of the inhibition of virus multiplication. The virus apparently reaches a high concentration before the "A system" of respiration ceases activity. Since the "B system," which is also cyanide-sensitive, seems to be little affected by the virus and continues to function beyond the cessation of virus multiplication, it is possible that the A component, or something connected directly with it, is necessary for virus multiplication. If the A system ultimately is inhibited or destroyed as a result of activity of the virus, the latter might thus bring about a condition preventing its own further increase.

A connection between certain oxidizing enzymes and virus multiplication already has been indicated by Woods (18, 19, 20) who has called attention to the relation of cellular oxidases and peroxidases to virus diseases of plants. While it is unlikely that the virus protein is, itself, of the nature of a peroxidase, there probably are fundamental relationships between multiplication of this protein and specific oxidizing mechanisms of the cell, as Woods indicated a number of years ago.

SUMMARY

Oxygen respiration in healthy Turkish tobacco leaves is accomplished by both cyanide-sensitive and cyanide-resistant systems of catalysts. The energy for protoplasmic streaming is derived from these respiration catalysts. Rate of streaming can be used as an index to cellular respiration.

There are two cyanide-sensitive systems, separable from each other by means of their "critical oxygen tensions"; these have been arbitrarily designated as the A and B systems. The third or "C system" is resistant to cyanide and continues to function at lower oxygen tensions than either the A or B components during progressive suffocation.

Infection with tobacco mosaic virus (*Marmor tabaci* H.) results in an inhibition in the A system 48 to 72 hours after infection of the cell. The B system apparently is not affected, but the C component is greatly increased in activity following inhibition of the A component.

The inhibition of the A system apparently occurs only after development of a high concentration of virus, and such inhibition of certain respiratory components may be the factor preventing further multiplication of the virus protein.

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A BACTERIAL NECROSIS OF THE GIANT CACTUS

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INTRODUCTION

Bacterial necrosis of the giant cactus (*Carnegiea gigantea* Britt. and Rose (*Cereus giganteus* Engelm.)), long present in southern Arizona, is a serious, hitherto undescribed[1] disease. Its recent encroachment on cactus parks and privately owned estates has made necessary the investigational work described in this paper.

IMPORTANCE

Bacterial necrosis has been observed in an area 200 miles broad, north and south, by 250 miles long, east and west. The disease is particularly destructive near Tucson, in Picacho Park, and near Maricopa; it occurs between Sacaton and Chandler, northwest of Phoenix, westward to Yuma, and along the Ajo highway from Tucson to the Mexican border. The malady varies in severity in different forests of giant cactus. For example, plants killed by necrosis plus living cacti with visible infection, on five 1-acre tracts in the Saguaro National Monument, amounted to 21, 0, 0, 4, and 18 per cent, respectively. The percentages on 3 similar tracts in the Santa Catalina foothills were 33, 13, and 9, respectively.

The figures on mortality and infection become serious when the comparatively rapid disappearance of the fallen fleshy cacti is considered together with the probability that many plants in the earlier stages of infection are missed in field reconnaissance. Most deaths counted probably occurred within the past 2 years, and many cacti in the forests studied are certainly condemned to death within a short time. Few seedlings are coming on to replace the dead and dying plants. It appears possible that bacterial necrosis may explain some growths of giant cactus in southern Arizona that are apparently on the way out (Fig. 1, *B*).

Bacterial necrosis is important economically, as well as botanically. The cactus that it destroys is an attractive feature for the many tourists that annually visit Arizona, and financial income from such visitors is greater than the income from many other sources. The disease has already invaded the estates of wealthy citizens who spend the winter in southern Arizona and whose requests for information on the necrosis have stimulated our studies.

[1] A brief note by Dr. Forrest Shreve of the Carnegie Desert Laboratory may have reference to bacterial necrosis: "When struck by lightning or wounded in any other manner during the dry season, it (the giant cactus) recovers very rapidly by the formation of a heavy callus over the wounded spot. If it is wounded in the rainy season, however, bacterial decay sets in very rapidly and a large plant may be destroyed in less than a week as a result of a small wound."—From Britton and Rose, The Cactaceae 2: 166. 1920.

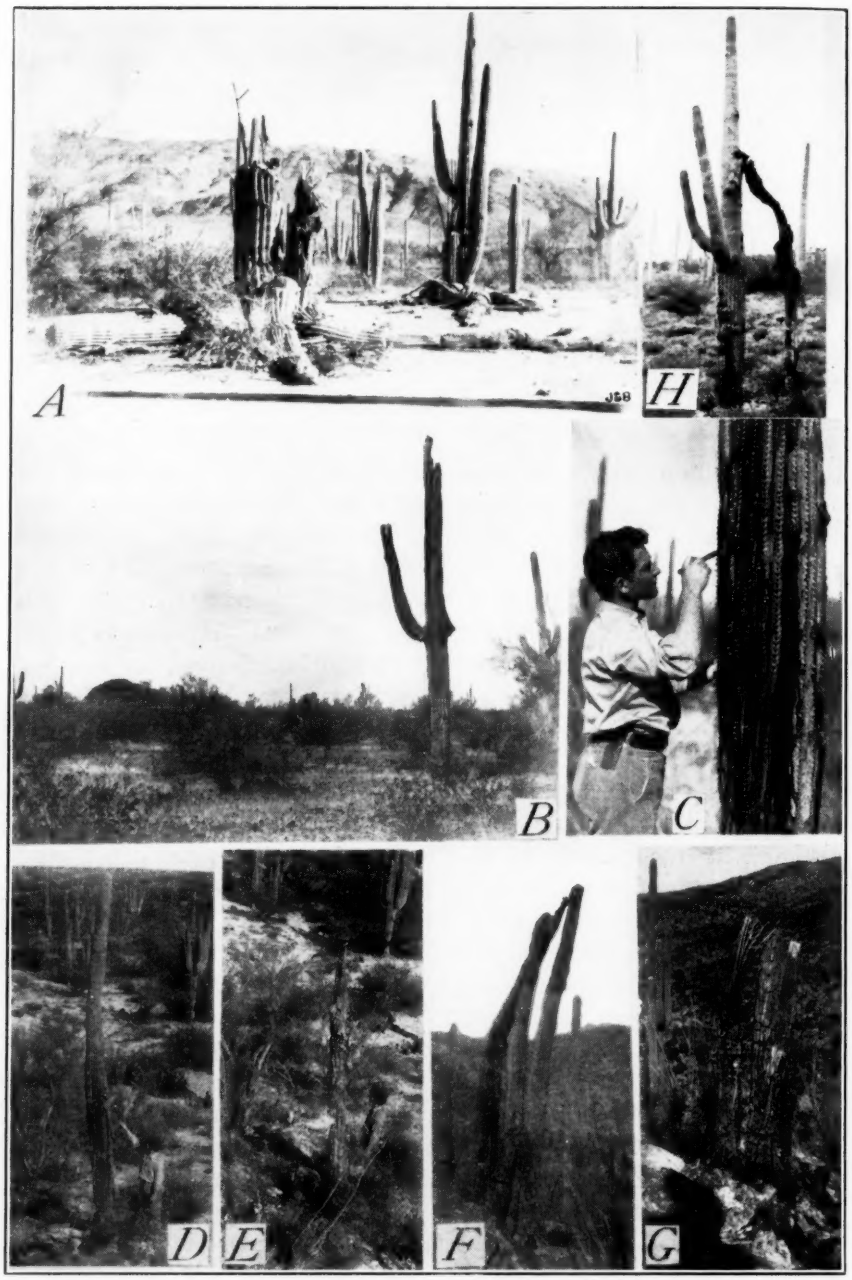

FIG. 1. *A*. Destruction of giant cactus, *Carnegiea gigantea*, resulting from bacterial necrosis. *B*. Thin growth of giant cactus believed to be mainly due to necrosis. Cactus in right foreground is "bleeding" at top and base. *C*. Close-up of part of trunk of infected cactus with streams of bacterial ooze; new stream below knife point. *D, E*. Rate of progress of necrosis: *D*, giant photographed February 10, 1940, and *E*, same plant November 2, 1940. *F*. Spread of disease in group: one cactus broken, a second is leaning against another plant; the two remaining cacti are badly infected. Taken February 10, 1940. *G*. Group of cacti illustrated in preceding photograph. Taken November 2, 1940. *H*. Spread of disease from one individual cactus to another. The decayed cactus fell against a branch of an adjacent plant to which it carried the necrosis.

SYMPTOMS

The first symptom of the bacterial necrosis of *Carnegiea* is a small, circular, light-colored spot, usually with water-soaked margin, on the surface of the integument of trunk and branches. Underneath the surface discoloration the soft parenchymatous tissues (Fig. 2, *A* and *B*) become water-soaked after bacterial invasion and subsequently brown to almost black. As the infection progresses the spot enlarges and assumes a purplish

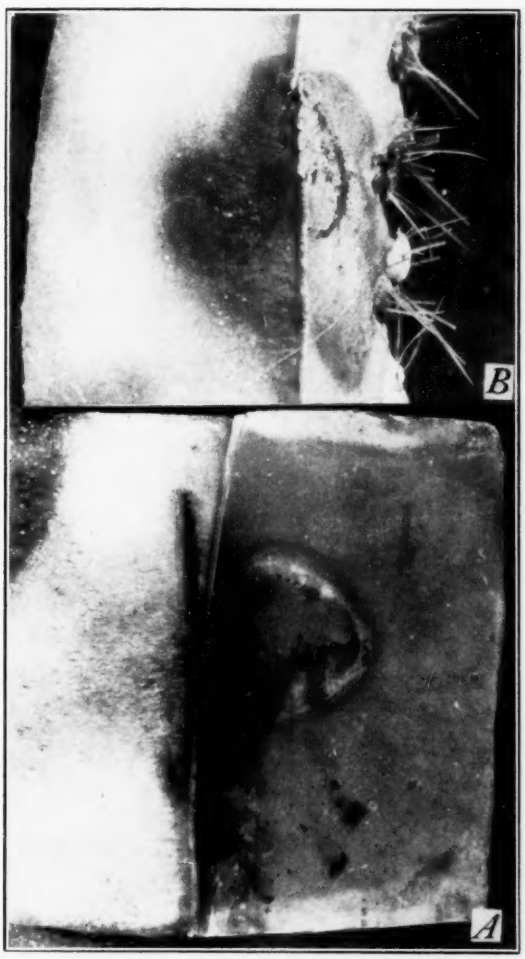

FIG. 2. Necrotic spots, *A*, natural, and *B*, artificial. Note discolored broken surface at right and darkened internal tissues, left. Elongated vertical streak in *A* is an artificial cut.

hue. In cases in which the infection advances rapidly the central part of the spot breaks (Fig. 1, *A* and *B*) and a brown liquid exudes. Rapid destruction of the tissues is then accompanied by "bleeding" (Fig. 1, *B*, *C*, and *H*) but slower internal decay may proceed without exudation. The rotted tissues dry, break up into granular to lumpy pieces and fall to the ground, leaving the woody stelar strands bare (Fig. 1, *A* and *G*).

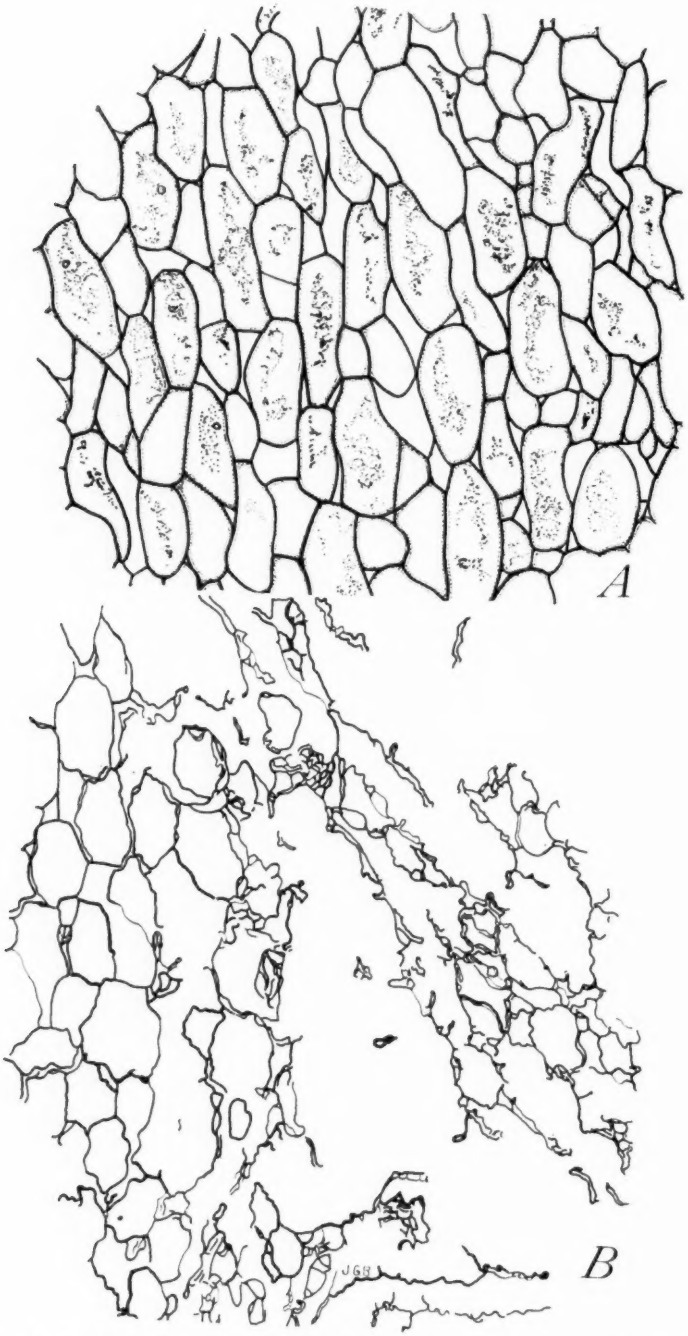

FIG. 3. *A*. Part of transverse section of healthy parenchyma consisting of turgid cells. *B*. Comparable section of necrotic tissues partly broken down with cavities. ×52.

Necrosis may start anywhere on the trunk and branches. Where it girdles the columnar trunk near the base, the plant is so weakened that the latter easily breaks in a windstorm. Such broken giants are only too common in the forests studied (Fig. 1, *A*). Decay at the top of the trunk leaves the woody ribs of the stele there exposed. Rotting on one side is followed by the leaning of the trunk toward that side (Fig. 1, *F*). Girdling of large branches at their bases causes the branch to sag and break off. Eventually the giant falls or, if it remains erect, the infected parenchyma disintegrates, exposing the bare woody skeleton. The necrosis kills quickly, often within 2 or 3 weeks, and in the course of a few months the affected tissues are almost completely broken down (Fig. 1, *E* and *G*).

HISTOLOGIC AND CYTOLOGIC CHANGES

Direct histologic and cytologic changes resulting from infection are limited to the integument (cuticle-covered epidermis and thick-walled hypodermal cells) and the underlying parenchymatous tissues, both within and without the stele. The drying, cracking, and discoloration of the cuticle-covered epidermis and the hypoderma have already been mentioned. The chlorenchyma loses its chlorophyll, becomes water-soaked and discolored brown to black; all other parenchymatous tissues likewise show water-soaking and discoloration. Finally, the soft tissues break down (Fig. 3, *A* and *B*) into a dark colored, almost odorless liquid unless slow destruction affords time for drying.

Cytologic changes affect the entire parenchyma cell. The wall loses its middle lamella by dissolution of the latter (Fig. 4, *H*, *I*, and *J*), so that the cells in transection appear to have a double wall separated by a narrow, more or less irregular space instead of a single, continuous wall. In other cases the unequal dissolving of the middle lamella leaves numerous cavities (Fig. 4, *I*) in the lamellar region. The remainder of the wall eventually becomes soft and irregular in outline (Fig. 4, *D*, *F*, and *J*). The disintegrating wall is not uniform in consistency, as shown by more persistent fibrous streaks (Fig. 5, *A* to *D*) in its mass. Quite early in the infected tissues nuclear changes are evident; the nuclear membrane becomes thickened (Fig. 4, *D* and *E*); the nucleoplasm loses its reticular aspect (Fig. 4, *C* to *G*) and becomes more or less homogeneous and retentive of stains. The nuclear mass may be considerably increased in bulk and the nuclear outline irregular and wrinkled; shrinking and disintegration follow. The nuclei of chlorenchyma cells become surrounded with chloroplasts (Fig. 4, E) that, like the former, assume an increased avidity for certain stains. The chloroplasts finally shrink and disappear.

The node of disintegration of the cell wall in bacterial necrosis deserves special emphasis in view of recent microchemical investigations (1) in which the walls of plant cells have been observed to consist of fibrils held together by a pectic substance. The fibrils are microscopic elongated particles of cellulose joined end to end. This fibrillar structure is evident in the

disintegrating cell walls in the giant cactus. Swelling of the pectic matrix (Fig. 5, *B* and *C*) separates and somewhat disarranges the fibrils. Dis-

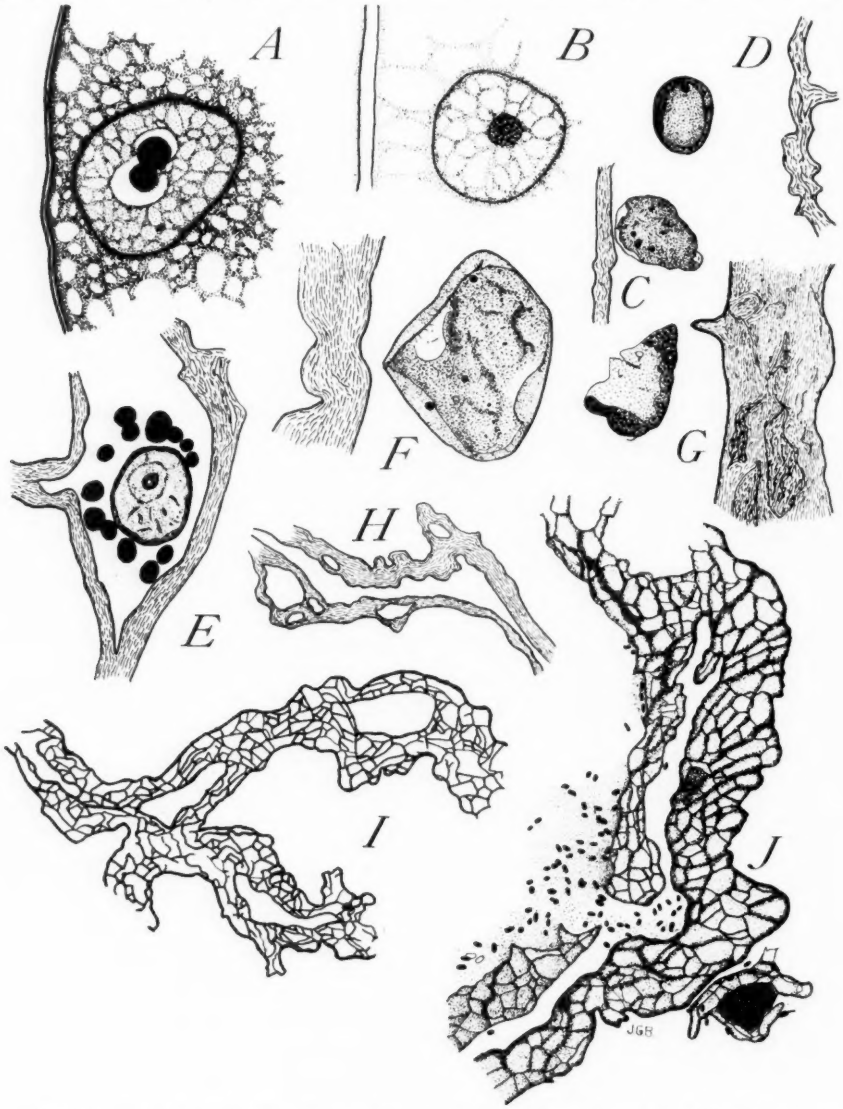

FIG. 4. Nuclear and cell wall studies. *A* and *B*. Normal nuclei and adjacent parts of cell wall. *C*. Nuclear reticulum lost; nucleus shrunken; wall somewhat swollen. *D*. Nuclear membrane thickened; wall considerably swollen and softened. *E*. Nucleus of chlorophyll cell with thickened membrane and with chloroplasts clustered around it. *F*. Disintegrating nucleus and much swollen cell wall. *G*. Later stage in disintegration of nucleus and in swelling of cell wall. *H*. Part of wall with middle lamella dissolved and cavities in outer layers of the wall. *I*. Later stage in dissolution of cell wall. *J*. Part of swollen cell wall with bacterial action in progress. All drawings made with camera lucida, ×1159.

solution of the middle lamella is usually first noticed, followed by the rapid breakdown of the matrix and less rapid dissolution of the fibrils.

Eventually, only a little of the wall remains (Fig. 5, *D*). Although like destruction of the walls of plants by a saprophytic soil bacterium has been described (2), similar stages in the disintegration of the walls of living plants through the action of parasitic bacteria appear to have been over-

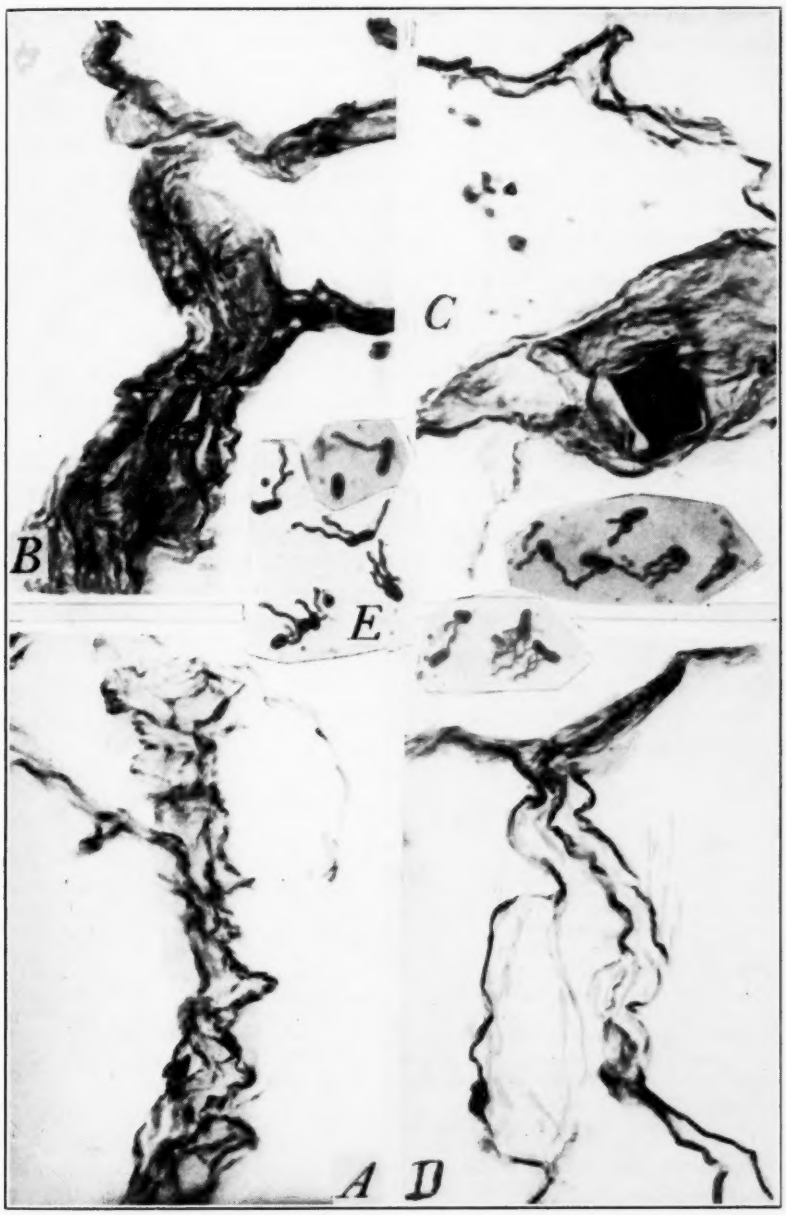

FIG. 5. Photomicrographs. *A*. Piece of swollen wall showing cellulose fibrils and pectic matrix. *B*. Fibrils separated by swelling of matrix. *C*. Later stage in swelling of cell wall, in center; wall above and below is almost gone. *D*. Last stage in decomposition of wall. *E*. Groups of the bacterium of necrosis. *A*, *B*, *C*, *D*, approximately × 1200.

looked by plant pathologists. They should be found in studies of the vegetable rots.

BACTERIOLOGICAL TECHNIQUE

Cubes of the diseased tissues of the giant cactus approximately 5 mm. on a side were cut out aseptically and either placed directly on agar slants or immersed in mercuric chloride solution (1:1000) for 1 min. before culture on agar. Some pieces of the tissues were incubated at room temperatures (22–37° C.) and other pieces at a constant temperature of 26° C. The pieces sometimes gave bacterial growth in 15 to 24 hr. but usually only after 3 or 4 days. Although stained samples of the colonies directly obtained from infected tissues often showed only one morphological form of bacterium, segregation by Koch's method (5) and by streak culture (4) was employed. From the resulting colonies of bacteria transfers were immediately made to potato-dextrose yeast-infusion agar and, from the latter, dilution plates were again poured. The pure cultures of bacteria thus obtained were identical, one culture with another, and also with pure cultures usually given by carefully surface-sterilized blocks of infected cactus tissues.

The bacterium from the necrotic tissues was cultured on 15 different kinds of solutions used as sources of carbon. For this work Dunham's tubes were used, each tube containing 10 cc. of nutrient broth, 0.5 per cent of the desired, separately sterilized carbon source, and 0.5 cc. of 0.4 per cent phenol-red solution. In addition, media consisting of 1 per cent and .2 per cent peptone-dextrose and 2 per cent peptone-levulose, respectively, in distilled water, were seeded with the bacterium. Other media commonly used in bacteriological laboratories, such as litmus milk, gelatin, lead acetate agar, plain nutrient broth, Uschinsky's solution, and malt-extract broth were employed in testing the bacterium from the necrotic giant cactus.

CHARACTERISTICS OF THE BACTERIUM

The bacterium (Fig. 5, *E*) that causes necrosis of the giant cactus is a greyish-white, actively motile, (15–24 hr., agar slants) peritrichiate (8), Gram positive (6), non-sporiferous, very slowly gelatin-liquefying (20° C.), nitrate-reducing, non-milk-curdling, aerobic short rod with rounded ends, occurring singly or in pairs, growing on the surface of agar poured plates in the form of round, slightly raised, smooth, grey-white wet-shining colonies having an entire, well-defined margin. Nutrient broth gave abundant growth. In Uschinsky's solution growth was moderate, liquid turbid, a slight ring appeared at the surface, and sediment was viscid upon agitation. Litmus milk was slightly pink or reduced with no curdling. There was no noticeable odor in any of the media. Acid and gas with abundant growth were produced with the following sources of carbon: arabinose, dextrose, galactose, levulose, maltose, sucrose, raffinose, mannitol, and salicin. For the bacterium is proposed the name **Erwinia carnegieana** Standring n. sp.

INOCULATIONS

Needle inoculations with **Erwinia carnegieana** n. sp. from numerous cultures, hypodermically made into trunk and branches of the giant cactus gave a straw-colored surface discoloration, like that observed in the field, in 3 days; in 5 days the spots were reddish-purple around the needle puncture, with a band of pale-yellow beyond; in 11 days the spreading

spots had a water-soaked margin. Internally, the parenchyma (Fig. 2, *B*) was water-soaked and brown to black, again like the change observed in nature. Sections of living and of killed, stained tissues from artificially inoculated giant cactus presented the same pathologic picture of histologic and cytologic changes previously described in this paper for the tissues of plants naturally infected in the field. From the artificially inoculated tissues was recovered the bacterium originally isolated from naturally infected tissues of the giant cactus and inoculated into the healthy plant.

LITERATURE REVIEW

No reference to a bacterial necrosis of the giant cactus, excepting the previously cited note, has been found in literature. At least two bacterial

TABLE 1.—*Comparative characteristics of B. cacticidus, B. cactivorum, and E. carnegieana*

Character	*Bacillus cacticidus*		*Bacterium cactivorum*	*Erwinia carnegieana*
	Liquid	Solid	Liquid and solid	Liquid and solid
Shape	Rods	Coccoid	Rods	Rods
Size	1.3 μ × 0.8 μ	0.8 μ in diameter	1.5 μ × 0.8 μ	1.12 μ–1.79 μ × 1.56 μ–2.90 μ
Grouping	Singly or in pairs		Not in chains	Singly or in pairs
Motility	Active		"Mobile"	Active (flagellate)
Gram's stain	Negative		Positive	Positive
Color	Dirty white to yellow		Not reported	White
Capsule	None		Not reported	Present
Spores	None		None	None

TABLE 2.—*Reactions*[a] *of B. cacticidus (4), B. cactivorum (7), and E. carnegieana n. sp.*

Media	*Bacillus cacticidus*			*Erwinia carnegieana*			Media	*Bacterium cactivorum*			*Erwinia carnegieana*		
	Acid	Growth	Gas	Acid	Growth	Gas		Acid	Growth	Gas	Acid	Growth	Gas
Arabinose	0	...	...	x	4	3	Peptone-dextrose, 1%	...	0	...	...	4	4
Dextrose	x	...	...	...	...	...	Peptone-dextrose, 2%	...	0	...	...	4	3
Galactose	...	...	...	x	4	3	Peptone-levulose, 1%	...	0	...	...	4	4
Levulose	...	...	...	x	4	3	Peptone-levulose, 2%	...	0	...	...	4	3
Lactose	0	...	...	0	3	0	Malt broth	x	3	x	0	3	0
Maltose	0	...	...	x	4	4							
Sucrose	x	...	...	x	4	4							
Dextrine	...	...	...	...	...	...							
Inulin	...	...	...	0	3	0							
Melezitose	...	...	...	...	...	...							
Raffinose	...	...	...	x	4	3							
Dulcitol	0	...	...	...	...	...							
Glycerol	...	...	...	0	4	0							
Inositol	...	...	...	0	4	0							
Mannitol	x	...	...	x	4	4							
Salicin	x	...	...	x	3	1							

[a] 0 = negative; x = positive; 1, 2, 3, 4, = arbitrary designations of increasingly abundant acid, growth, and gas production.

rots, however, are described that affect cacti belonging to other genera. One rot with symptoms somewhat like those occurring in *Carnegiea* was studied by Pasinetti and Buzzati-Traverso (7) in *Cephalocereus senilis* in Italy. The rot was attributed to a bacterium isolated from the decaying tissues and proved pathogenic by inoculation; it was named *Bacterium cactivorum* Pasin. et Buzz.-Tr. sp. nov. The second rot, investigated by Johnston and Hitchcock (4), was found in flat opuntias growing in Florida. From the sick plants was obtained a bacterium pathogenic by inoculation in 7 species of prickly pear. This bacterium was designated *Bacillus cacticidus* Johnston and Hitchcock n. sp. A summarized comparison of the two bacteria with the bacterium from the giant cactus is given in tables 1 and 2.

DISCUSSION

The rot of *Cephalocereus senilis* described (7) by Pasinetti and Buzzati-Traverso is characterized by symptoms not unlike those of the necrosis of the giant cactus. In both diseases a dark discoloration accompanies the softening of the infected tissues. The decay in *Cephalocereus,* however, spreads so constantly from the base of the plant toward the apex that it has been termed "humid basal rot"; in *Carnegiea* the rot proceeds from the infected spot in any direction that the parenchyma makes possible. Nor does the description of the pathogen, *Bacterium cactivorum,* agree with that of the pathogen, *Erwinia carnegieana.* The former, much smaller organism grows neither in peptone-dextrose nor in peptone-levulose and forms gas in malt broth; the reactions of the giant-cactus bacterium in those media are just the opposite. The limited extent of the reactions reported for *Bacterium cactivorum* make further comparison impossible; the data given for the pathogen are sufficient, we believe, to separate the two organisms.

The rot of the genus *Opuntia* attributed to the activities of *Bacillus cacticidus* is still more unlike the necrosis of the giant cactus than the rot of *Cephalocereus,* although, so far as symptoms are concerned, they are both "wet" rots. The pathogen isolated from the infected opuntias is smaller than *Erwinia carnegieana,* coccoid on solid media, gram negative, and may become yellow. More than 30 inoculations of *E. carnegieana* into *Opuntia* spp., hypodermically made at different times, failed. Moreover, opuntias abound in close proximity to necrotic giant cacti, yet none affected by a similar disease has been observed by us in the groves of southern Arizona. These conflicting data appear to be sufficient to remove the pathogen from our consideration as the cause of necrosis of the giant cactus.

Finally, the necrosis of *Carnegiea* and its pathogen, as well as the succulent nature of the host, remind the pathologist of vegetable soft rot and its cause, *Erwinia carotovora.* Both diseases affect parenchyma, both involve the dissolution of the middle lamella of the cell wall, both progress rapidly at high temperatures, and there are other similarities. However, the two pathogens differ in several respects,—size, grouping in cultures, usual reaction to Gram's stain, in milk, and in some carbon-source

media. Cross-inoculation of the giant cactus with *Erwinia carotovora* gave negative results and numerous repeated inoculations of carrots and other hosts of the soft-rot organism with *Erwinia carnegieana* were likewise negative.

SUMMARY

A bacterial necrosis of the giant cactus (*Carnegiea gigantea* [*Cereus giganteus*]) is killing varying numbers of that plant over an extensive area in southern Arizona.

That the thinning of cactus groves now in progress may have been repeated in the past and may have continued until the affected groves became sparse or actually disappeared is suggested.

Macroscopic and microscopic features of the necrosis are described, also the cause, and a name is proposed for the pathogen.

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PHYTOPHTHORA PARASITICA ON PAPAYA (CARICA PAPAYA) IN HAWAII[1]

G. K. Parris

(Accepted for publication June 25, 1941)

INTRODUCTION

In March, 1940, a fruit- and stem-rotting disease of papaya was observed at Kahaluu and Kailua on the island of Oahu. At the former locality the disease was attacking approximately 40 per cent of the trees in a 4-acre planting and causing the death of the bearing portions of some 25 per cent. At Kailua, less damage occurred.

The cause of the disease was identified as *Phytophthora parasitica* Dastur, a diagnosis confirmed by C. M. Tucker.[2] A survey of the literature revealed that no investigator has presented a complete picture of Phytophthora rot of the papaya plant, and in only a few cases have Koch's rules of proof been applied to the causal agent.

LITERATURE REVIEW

Two species of *Phytophthora* have been reported attacking papaya in nature, *P. parasitica* (1, 11) and *P. palmivora* Butler (2, 3, 5, 6, 7, 8), and several unidentified species also have been recorded for this host. Tucker (10) determined the pathogenicity to papaya of a number of species of *Phytophthora*, including the two mentioned above, and found that not all strains of the same species were pathogenic and that a difference in pathogenic virulence existed between different strains. Mehrlich (4), Tucker (10), and Thung (11) showed that strains of *parasitica* would infect papaya only through wounds, and most investigators agree that infection by *palmivora* also takes place through wounds. Gadd (2), however, found that wound-free papaya fruits were infected by strains of the fungus from papaya and from rubber, while strains from other plants produced infection only after wounding.

SYMPTOMATOLOGY

Morphologic Symptoms. The terminal leaves on a diseased plant droop, wilt, dry out, or become yellowed, and fall prematurely. Eventually, only a few under-developed leaves persist at the apex of the plant. Fruits on a diseased plant may, also, fall prematurely. These symptoms (Fig. 1, A) are not unlike those exhibited by papayas suffering from foot (collar) rot or root rot, caused by various species of *Pythium*, particularly *P. ultimum* Trow (9, 12). In Hawaii root rot is caused by *P. aphanidermatum* (Eds.) Fitzpatrick.[3] Collar rot is infrequently caused by *Phytophthora parasitica*, but

[1] Published with the approval of the director as Technical Paper No. 86 of the Hawaii Agricultural Experiment Station.

[2] Personal correspondence of December 19, 1940.

[3] Identified by J. T. Middleton; personal communication.

the roots of the papaya have not been observed to be attacked and diseased plants are firmly anchored in the soil. However, it should be noted (Table 1) that papaya roots are susceptible to the latter fungus, particularly when wounded.

With the disease under discussion, the symptom complex on the aerial parts of the plant results from one or more stem cankers, usually located in the upper third of the length of the stem. In their initial stages the cankers are small, $\frac{1}{2}$ to 1 in. in diameter, water-soaked, darker than normal, ovate to circular, and usually partly or completely covered with a whitish crust or mildew. The closely packed fruits on the stem of a bearing papaya tree

FIG. 1. Papaya trees diseased with *Phytophthora parasitica*. A. Top of plant killed following severe infection of stem in fruit-bearing region. Ground beneath tree shows numerous mummified fruits. B. Close-up of cankers on stem of less-severely affected tree than shown in A; note that many fruits and leaves have fallen prematurely and that the remaining foliage is wilting. Several fruits show white patches of mildew.

may hide the cankers for a while, but as the cankers enlarge, fruits in their vicinity are attacked and fall, leaving one or more portions of the stem conspicuously bare (Fig. 1, B). The texture of a canker is rubbery, seldom soft; latex may or may not ooze from around the edges. With aging, the canker enlarges in both directions, but more rapidly along the length of the stem, until it may occupy an area of approximately 6″–8″ × 2″–4″. On a small plant, such a canker completely girdles the stem; on an older plant the stem above the lesion is seldom rotted or killed by the pathogen, but it is so structurally weakened that, in wind, it usually snaps off at the cankered zone.

TABLE 1.—*Results obtained following inoculation of wounded and unwounded papaya leaves, stems, fruits, and roots with Phytophthora parasitica*

Point of inoculation		With or without wounding	With or without benefit of a moist chamber for 12 hours	Number of plants, or plant parts, inoculated	Number of plants, or plant parts, diseased	Type of infection		Number of infections from which fungus recovered
						Severe	Mild	
Stem	Upper third of length	Without	Without	4	0	.....	.....	.....
			With	5	3	.....	3	1
		With	Without	5	5	5	.....	4
			With	1	1	1	.....	1
	Middle of length	Without	Without	7	0	.....	.....	.....
			With	5	5	.....	5	2
		With	Without	6[a]	3	1	2	2
			With	1	1	1	.....	1
	Lower third of length	Without	Without	5	0	.....	.....	.....
			With	5	1	.....	1	0
		With	Without	5	4	4	.....	3
			With	1	1	1	.....	0
Leaf	Upper surface	Without	Without	5	0	.....	.....	.....
			With	5	5	5	.....	1
		With	Without	5	5	.....	.....	.....
			With	5	5	1	.....	1
	Lower surface	Without	Without	5	0	.....	.....	.....
			With	5	0	.....	.....	.....
		With	Without	5	0	.....	.....	.....
			With	5	1	.....	1	0
Root		Without	Without	7	0	.....	.....	.....
			With	4	1	.....	1	1
		With	Without	6	1	.....	1	0
			With	5	5	4	1	3
Fruit		Without	Without	10	0	.....	.....	.....
			With	10	7	7	.....	5
		With	Without	10	10	10	.....	7
			With	10	10	10	.....	8

[a] Five of these six plants were inoculated on wounds created by purposely breaking away a petiole of an old leaf.

As mentioned above, this disease infrequently causes cankers at the collar of the plant, that is immediately above the soil line. Here the organism meets with more resistance than when the stem proper is attacked. Even though an appreciable area may be killed, the plant usually survives but shows a semi-open cavity filled with dry, coarse fibres—the shredded and digested tissues of the stem. Immature plants may succumb to the collar-rot phase of this disease.

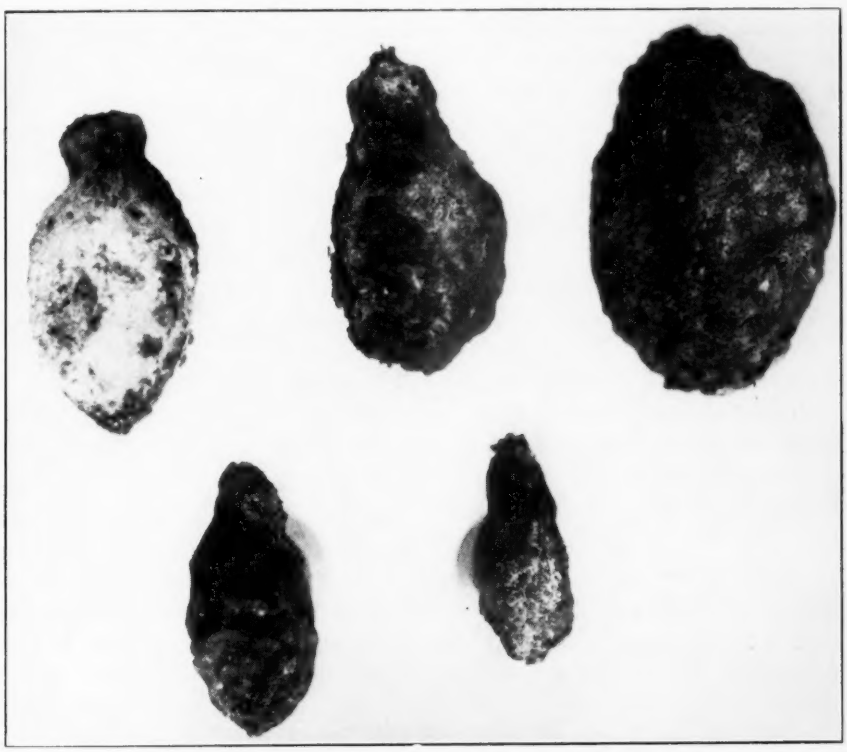

FIG. 2. Papaya fruits diseased and mummified following field infection by *Phytophthora parasitica*. Note the mycelium of the fungus fruiting on the surface of the mummies. Mummies are one-third to one-fifth size of originally healthy fruits.

Unlike the more common anthracnose rot of papaya fruits, caused by *Colletotrichum* sp. (probably *C. gloeosporioides*), Phytophthora rot causes the fruit to slowly shrivel, harden, and turn grayish-green. The surface may show one or more patches of a whitish incrustation, reminding one of powdery mildew (*Oidium* sp.). The mummified fruits ultimately become brownish-black (Fig. 2), light in weight, and of a stone-like texture. Fruits of all ages may be attacked.

Leaves were not observed to be diseased in the field, but were susceptible to artificial inoculation (Table 1).

Histologic Symptoms. Mycelium in diseased fruits is both inter- and intracellular. In newly diseased fruits circular, thin-walled, densely proto-

[a] Five of these six plants were inoculated on wounds created by purposely breaking away a petiole of an old leaf.

plasmic structures are found, sometimes attached to strands of mycelium (Fig. 3, A); in mummified fruits, oval or irregularly rounded chlamydospores with thicker walls are present (Fig. 3, B, C).

ETIOLOGY

The cause of this disease is *Phytophthora parasitica* Dastur, which produces its ovate, colorless, papillate, sporangia, attached to the host by short

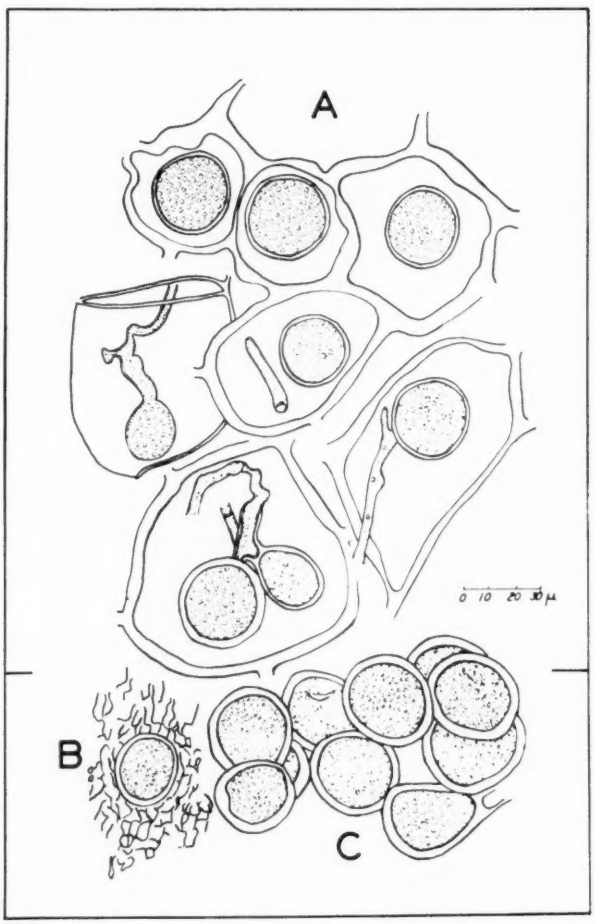

FIG. 3. Chlamydospores of *Phytophthora parasitica* in tissues of diseased papaya fruits. A. In freshly diseased fruits. B and C. In mummified fruits. In B the disorganized host tissue is pictured.

sporangiophores, on the surface of diseased parts. Placed in tap water at room temperature (27–28° C.), the sporangia germinate after the manner of a *Phytophthora*. One hundred sporangia from diseased fruits measured 32.0–38.4 μ by 22.4–25.6 μ, closely approximating dimensions given by Tucker (10) for *P. parasitica*. This worker has reported that *P. parasitica*, unlike *P. palmivora*, will grow at 35° C.; though it is significant to note that

Mehrlich (4) found that certain strains of these species, isolated from pineapple in Hawaii, could not be separated from each other by growth or lack of growth at this temperature. Held at $35.5 \pm 0.5°$ C. for 96 hours, the papaya isolate made no growth but the fungus was not killed; replaced at room temperature, all cultures soon developed abundant mycelium. Tucker[4] found that the Hawaiian isolate grew at 35° C.

Pathogenicity. The fungus was isolated in pure culture from diseased fruits and stems, and papaya leaves, stems, fruits, and roots were inoculated, with and without wounding, and with and without benefit of a moist chamber for 12 hours following inoculation. Wounds on stems, fruits, and roots were produced by a sharp scalpel; a small portion of plant tissue was removed, mycelium from an agar culture inserted, and the tissue replaced. Leaves were wounded by scarification with a needle. Following inoculation, plants were placed in a greenhouse where the temperature ranged from 75° F. (night) to 90° F. (day). In no case did any check plant, wounded or not, become diseased. Inoculations performed and the results obtained are presented in table 1. When infection occurred, attempts were made to reisolate and identify the pathogen; in many instances this was done, thus completing Koch's rules of proof.

No infection of uninjured parts occurred unless the plant was kept in a moist chamber following inoculation. In the absence of a moist atmosphere for a prolonged period, infection occurred readily when the fungus was introduced *via* a wound, except in the case of the lower surface of the papaya leaf where the concurrence of wounding and a high humidity was necessary for infection.

Young stems were girdled in 7 to 9 days, and fruits were covered with sporangia in 10 and mummified in 30 days, after inoculation. The appearance of leaf lesions was typical of Phytophthora blights; in 4 days the inoculated areas were necrosed, but there was no subsequent enlargement. The lower surface was more resistant than the upper surface of the leaf. Inoculated leaves abscissed prematurely. Root infection was relatively slow to affect the plant as a whole. After 2 to $2\frac{1}{2}$ weeks, the leaves of inoculated plants fell prematurely and the stem was anchored less securely in the soil than is normally the case. Subsequently, plants inoculated on the roots toppled over or could be easily uprooted. The tap root (inoculated) was softened and somewhat decomposed and few laterals were alive. These plants often died in another week to 10 days, but sometimes the plant slowly recovered and continued to grow with the stem in a semi-recumbent position. Inoculations at the collar produced a similar response.

CONTROL

Growers decapitate trees as soon as cankers appear on the stems, and collect and destroy diseased material. Decapitation saves time; such trees produce fruits sooner than do replants. Sooner or later, a certain per-

[4] Personal correspondence.

centage of the new shoots that develop are diseased, and the decapitation procedure is repeated.

Spraying the papaya plant with a copper fungicide undoubtedly would control Phytophthora rot, but, so far, no attempt has been made to protect the plants. Local data have shown that Bordeaux mixture causes russetting of fruits, burning of the young foliage and a stunting of the papaya plant. Cuprocide 54–Y causes no comparable damage.

SUMMARY

A disease of papaya (*Carica papaya*) in Hawaii, which causes hard rotting of fruits and cankers on the stem, is described. Diseased fruits fall prematurely and mummify, while cankered stems may be girdled and distal parts die from lack of water and nutrients; or the stem may snap off in the wind at the cankered zone. Symptoms on the plant as a whole resemble those of root rot of papaya (*Pythium* spp.); but, in the field, the roots are not thought to be attacked by the cause of the disease, which is *Phytophthora parasitica* Dastur.

The fungus is essentially a wound parasite, though it will infect wound-free stems, fruits, roots, and leaves if the plant be kept in a moist chamber following inoculation. In a large number of instances the fungus was recovered and identified from artificially induced lesions.

Growers decapitate diseased trees, since beheaded plants produce fruits sooner than do replants. A copper fungicide probably will control the disease, but has not been tried. Bordeaux mixture russets papaya fruits, burns young foliage, and stunts the plant; Cuprocide 54–Y does not produce these harmful effects.

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THE EFFECT OF TEMPERATURE ON SYMPTOM EXPRESSION OF A ROSE MOSAIC[1]

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The expression of symptoms of rose mosaic is recognized as being erratic under even favorable environmental conditions (2, 3, 9). Symptoms may be exhibited in some leaves or leaflets, while adjacent ones frequently are normal in appearance. It is practically impossible accurately to diagnose the disease in named varieties, growing outdoors, because of the mildness or lack of leaf symptoms and the presence of injuries from insects, fungi, chloroses, and genetical leaf markings (2, 3, 6). In rootstock varieties the situation in the field is even worse. There is no convincing evidence that field diagnosis of this mosaic in rootstock or bud varieties is possible on a commercial scale.

Plants, apparently mosaic-free when growing outdoors, may develop striking symptoms when moved into the greenhouse; but leaves formed after being returned outdoors will be normal. On April 4, 1940, 12 plants of each of 2 varieties, Peerless and Rome Glory, all of which showed typical symptoms in the glasshouse, were pruned and defoliated before being moved outdoors. Positive symptoms were not shown by any of the abundant new leaves produced during the next 5 months. Two plants of each variety, handled similarly but kept in the glasshouse, continued to show typical symptoms. White (8) reported similar observations.

Temperature is one of the environmental factors that may be involved in this difference in symptom expression outdoors and under glass. This relationship was investigated in studies reported in the present paper.

MATERIALS AND METHODS

Infected plants of the Peerless and Rome Glory varieties were selected in commercial greenhouses where they had been growing $2\frac{1}{2}$ to 3 months. These plants were placed in 10-inch pots and transferred to a greenhouse in Berkeley for 37 days to recover from transplanting shock. Each pot was mulched with well-rotted manure and, during the experiments, occasionally watered with a dilute solution of potassium sulphate and ammonium sulphate.

The 12 plants of each variety that showed most conspicuous symptoms were transferred to 2 controlled-environment chambers[2] for the experiment. In the 3 series of tests the atmosphere was held at a saturation deficit approximately equal to a relative humidity of 70 per cent at 17° C. (*i.e.*, 4.3

[1] This study is limited to the disease which produces symptoms of the type commonly seen in greenhouses (2, Figs. 1 and 2; 3, Figs. 1, A, and 1, C; 6, Fig. 1; 9, Figs. 1, B, and 3, A).

[2] Equipment was elaborated and improved by Davis and Hoagland over that described in an earlier paper (4), and was kindly made available for these studies by the Division of Plant Nutrition, University of California.

g. water per cu. m. of air would produce saturation at 17° C.). The plants received no sunlight, but were illuminated for 10 hours each day with 20 24-inch fluorescent tubes inside each chamber, 13 being pink and 7 blue. Because growth was somewhat spindling under these conditions in the first series, supplemental illumination of 4 incandescent 300-watt bulbs with reflectors was placed outside each glass-walled chamber during series 2 and 3. Duration of the daily light period also was 10 hours in these series.

In series 1 the 2 chambers were held continuously for 37 days at 17° and 25° C., respectively. The latter temperature under these constant conditions was too high for good growth of these varieties and caused partial defoliation. However, plants from these 2 temperature tests showed approximately equal growth in the next series. In series 2 one chamber was operated at 25° C. for the 10-hour light period and at 19° C. during the dark period. The other chamber was operated similarly at 18° day and 12° C. night temperatures. In series 3 one chamber was kept continuously at 21°, while the other was alternated from 21° day to 15° C. night temperature. The duration of the second series was 56 days and of the third, 62 days. The plants were redistributed in the chambers at the beginning of the second and third series to compensate for possible effects of previous environment. However, no such effects were noted on either symptoms or growth.

At the beginning and end of each series separate counts of mosaic and normal leaves, and measurements of growth were made on each shoot. These are presented in table 1 for each of the series.

TABLE 1.—*Effect of temperature on symptoms of rose mosaic in the Rome Glory and Peerless varieties*

Variety	Series	Temperature (°C.)		Shoot growth (cm.)	New leaves	
		Night	Day		Number	Mean per cent with mosaic[a]
Rome Glory	1 a	17	17	124.0	112	44.53 ± 5.24
	b	25	25	165.3	124	36.48 ± 4.33
	2 a	12	18	146.1	113	26.79 ± 4.58
	b	19	25	356.3	140	40.38 ± 3.57
	3 a	15	21	442.0	175	13.58 ± 2.98
	b	21	21	937.2	349	27.67 ± 2.76
Peerless	1 a	17	17	244.1	137	63.99 ± 4.07
	b	25	25	106.6	89	56.19 ± 6.37
	2 a	12	18	200.5	179	18.27 ± 2.77
	b	19	25	181.6	113	41.08 ± 5.23
	3 a	15	21	962.1	266	35.73 ± 3.81
	b	21	21	690.3	200	51.53 ± 3.69

[a] Estimated standard error of the mean was computed by the formula,

$$\sigma_x = \frac{1}{n}\sqrt{\frac{p_1q_1}{m_1} + \frac{p_2q_2}{m_2} + \ldots \frac{p_nq_n}{m_n}},$$

in which n is the number of plants (6 in each case), m the number of new leaves on a given plant, p the probability of a leaf showing mosaic symptoms, and $q = 1 - p$. Since these true probabilities, p and q, are unknown for a particular plant, the estimates based on observations are the best substitutes for them. In this analysis p and q were expressed as the percentage of mosaic and normal leaves, respectively.

RESULTS AND DISCUSSION

In these tests Rome Glory exhibited consistently better growth at the higher of the two temperatures in each series, and Peerless showed better growth at the lower range, although the varieties were intermingled and grew under very similar conditions. Despite this dissimilar growth trend, there was definite consistency in the relationships of symptom expression and temperature exhibited by the two varieties. This suggests that the response to temperature is a result of action on the virus rather than on the host. Beyond the minimum requirement of production of some vigorous new leaves, there was no observed relation between vigor of growth and severity of symptoms exhibited.

In series 1 the symptoms at 17° were strong and leaflets often were badly distorted, with conspicuous white markings; those at 25° C. were less severe. In series 2 the symptoms in both varieties were definitely suppressed in the low-temperature chamber of 12° and 18° C. In the third series symptoms were likewise suppressed in the chamber at 15° and 21° C. Because the severity of the symptoms was observed to increase with the percentage of leaves affected in any given series, the latter numerical index of disease severity was used.

Plants of the Peerless variety grown at 12° at night and 18° C. during the day, and those on a 15° and 21° C. schedule, had markedly lower percentages of mosaic leaves than the plants grown at the other temperatures (Table 1). Rome Glory grown at a night temperature of 15°, and 21° C. during the day, had significantly lower percentages of mosaic leaves than those held at other temperatures, and the trend was the same for those on the 12° and 18° C. schedule, although the differences were not so great.

There was considerable fluctuation in percentage of mosaic leaves in other series, but the levels were higher than for the two series mentioned above. It is quite possible that, with larger populations of plants, a more definite optimum for expression of mosaic symptoms would be found, but the important point here is that unmistakable symptoms are produced over a considerable range of temperatures. The severity and incidence are reduced conspicuously only by temperatures of 15° C. or less; but these temperatures apparently need not be continuous to be effective.

Even under the carefully controlled environmental conditions of the series held continuously at a favorable temperature for symptom expression, there was no consistency in the distribution of leaves showing the disease. A given leaf adjacent to another that showed mosaic might or might not exhibit symptoms, and even companion leaflets were frequently dissimilar in this respect. The virus is either extremely sensitive to the micro-environment of the leaf, an unlikely condition in view of the range of favorable temperatures, or some unknown factors in the physiology of the leaf or the movement of the virus are involved.

The inhibiting effect of low temperatures on the mosaic may account in part for the usual absence of symptoms outdoors, but probably is not the

sole factor involved. Under coastal California conditions the temperature during the active growing season sometimes stays within the limits for expression of symptoms for periods of 2 or 3 weeks, but the disease is almost never seen in the field.

Roses usually are grown commercially in glasshouses at temperatures of 14.5°–17° C. at night and, when possible, 20°–25° C. in the daytime. These ranges are such that temperatures under glass would be almost continuously conducive to expression of symptoms. For that reason it is possible to identify vigorously growing infected plants with certainty in the glasshouse and, if desired, rogue them out.

RELATION OF SYMPTOM EXPRESSION TO CONTROL OF THE DISEASE

Roguing in a glasshouse is justifiable if the flowers of the variety are sufficiently injured or reduced in number (3, 10) that the infected plants do not return a profit. The plants adjacent to those removed will benefit from the increased space, light and lessened soil competition, or if roguing is done early the beds can be replanted.

There apparently is little reason to fear that the disease will spread under glasshouse conditions, and infected plants need not be removed because of that consideration alone. As early as 1930 White (8) stated that "Due to its apparent failure to spread in the Eastern greenhouses, the commercial grower has rogued out diseased individuals very successfully." Beds having up to 50 per cent of infected plants in 1928–29 were rogued in the summer of 1929, and in 1930 diseased plants were either absent or present only in very small numbers. He later (10) found that 8 out of 36 seemingly healthy plants in a greenhouse test developed mosaic in the first 3 months, but no more appeared in the following 21 months. Presumably growth was insufficiently vigorous in the first 3 months for reliable diagnosis. Berkeley

TABLE 2.—*Incidence of mosaic in several rose varieties in glasshouses at two dates, as a measure of spread of the disease*

Variety	Number of plants	Initial age of planting	Interval between observations	Per cent plants with mosaic	
				Initial	Final
		Months	*Months*		
Better Times[a]	84	22	9	22.6	22.6
Peerless[b]	204	11	9	13.2	13.7
Hollywood	119	24	25	16.0	16.8
Rome Glory	70	2½	8	42.9	47.1

[a] Examination made 4 months after planting showed 11.9 per cent mosaic; this reading was later found to be inaccurate, as some of the plants had showed insufficient new growth for accurate diagnosis.

[b] Three months after planting had only 7.4 per cent mosaic detectable for the same reason indicated for Better Times.

(1) found that after removing the infected 50 per cent of Premier rose plants in a Toronto greenhouse, "very few diseased plants were observed after 3 months' time."

General observations and successive counts in California commercial glasshouses at intervals up to 2 years have given no clear evidence of the spread of rose mosaic. These data are summarized in table 2. The slight increases noted are explainable by the fact that the growth status of some of the plants at the time of early observations made accurate diagnosis difficult; this is shown in the readings taken 2½–4 months after planting. The disease certainly cannot be regarded as dangerously infectious in greenhouses and this interpretation is reflected in the present attitude of commercial growers toward it.

It is interesting that the disease, practically from the time of its first clear recognition, has been regarded as not spreading in the glasshouses. However, until comparatively recently (2, 3, 6), the prevalent view has been that natural spread did occur in the field (5, 7, 8). Because of the admitted difficulties of field, as compared with glasshouse, diagnosis this situation is not hard to understand when the relative dependability and severity of symptoms in the two locations are considered. However, as pointed out by Brierley and Smith (2, 3), the nursery practices of propagating understocks from the tops of previously budded plants, and of using budwood from top varieties growing outdoors, probably account for known field "spread" of the disease. When buds are taken in glasshouses, there is the hazard that they will be taken from the larger, nonproductive, mosaic-infected plants rather than from the much-pruned healthy ones, particularly if the nature and appearance of the disease are not understood (6).

For varieties that suffer flower deformation or yield reduction from the disease, it would be well to select budwood from greenhouse plants that have been examined at intervals and found mosaic-free. Before starting large-scale propagation of a new greenhouse variety, nurserymen could well grow the plants for budwood under glass at temperature favorable for the detection of the disease. This selected material could then be increased in mother blocks in the field.

Whenever necessary to increase the supply of budwood in the field it would be a reasonable precaution to plant at some distance from any infected nursery stock.

SUMMARY

Symptoms of mosaic were found in tests with two rose varieties growing under conditions of controlled environment to be favored by temperatures of 15–25° C. and to be greatly reduced in severity only below 15° C.

Under carefully controlled environmental conditions, as under fluctuating glasshouse situations, there was an erratic appearance of symptoms in comparisons between adjacent leaves or companion leaflets.

The response of the varieties to temperature was consistently distinct in the matter of growth, but in mosaic symptom-expression it was the same.

Because of the wide limits of the favorable temperature range for mosaic symptoms, it should be possible for certain purposes to grow satisfactory

budwood under glasshouse conditions, where diseased plants can be recognized and eliminated, and where the danger of new infections is slight.

There was no evidence of spread of the mosaic in commercial greenhouses.

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PHYTOPATHOLOGICAL NOTES

Pythium arrhenomanes on Cereals and Grasses in the Northern Great Plains.[1]—On the basis of pure-culture isolations made from field-grown cereals and grasses in North Dakota in 1940 and 1941, the common root-browning organism, *Pythium arrhenomanes* Drechsl., appears to be prevalent. While it seems to be particularly important in the lighter soils of central North Dakota, it is also abundant in the Red River Valley and has been isolated from the northern, extreme western, and northeastern parts of the State with frequent regularity. Isolations have been made also from wheat, corn, and certain grasses in adjacent Minnesota and South Dakota, and from wheat in eastern Montana. Strains of *P. aristosporum* Vanterpool, another species strongly pathogenic to cereals and grasses, which is occasionally isolated from wheat affected with browning root rot in Saskatchewan,[2] were obtained from barley roots from Dickinson, North Dakota, and from wheat roots from Bozeman, Montana.

During 1941, the disease first appeared about May 10, on various hosts, and continued to develop until about July 10. *Pythium arrhenomanes*, however, was isolated from such warm temperature grasses as *Setaria italica* (L.) Beauv. until early August, and in the Red River Valley was isolated from wheat as late as July 29. Other species of *Pythium*, including *P. debaryanum* and several congeneric forms, were isolated also from damping-off grasses early in the season and, to some extent, later, following the rainy periods.

Pure cultures of *Pythium arrhenomanes* isolated from a number of hosts proved parasitic on Thatcher wheat seedlings grown under aseptic conditions in small Erlenmeyer flasks held at room temperatures at Saskatoon, in August, 1941, and at Mandan, N. Dak., in September, 1941. Cultures from the following field-grown, naturally infected hosts were moderately to severely parasitic on the wheat seedlings: *Aegilops triuncialis* L., *Agropyron amurense* Drob., *A. caninum* (L.) Beauv. (the seed of this lot came originally from Russia and appears to be true *A. caninum* and not *A. caninum* Amer. Auth., which latter, according to Hitchcock, is *A. subsecundum*), *A. ciliare* (Trin.) Franch., *A. cristatum* (L.) Gaertn. (standard), *A. cristatum* (rhizomatous form), *A. dasystachyum* (Hook.) Scribn., *A. intermedium* (Host) Beauv., *A. pungens* (Pers.) Roem. and Schult., *A. repens* (L.) Beauv., *A. trachycaulum* (Link) Malte (*A. pauciflorum*), *Ammophila arenaria* (L.) Link, *Bouteloua curtipendula* (Michx.) Torr., *B. gracilis* (H.B.K.) Lag., *Brachypodium sylvaticum* (Huds.) Beauv., *Bromus carinatus* Hook. and Arn., *B. erectus* Huds., *B. inermis* Leyss., *Echinochloa crus-*

[1] Cooperative investigations between the Divisions of Cereal Crops and Diseases, Forage Crops and Diseases, and Dry Land Agriculture, Bureau of Plant Industry; and the Division of Nurseries, Soil Conservation Service, U. S. Department of Agriculture; the North Dakota Agricultural Experiment Station; and the Plant Pathology Laboratory of the University of Saskatchewan, Saskatoon, Canada.

[2] Vanterpool, T. C. Present knowledge of browning root rot of wheat with special reference to its control. Sci. Agr. **20**: 735–749. 1940.

galli (L.) Beauv., *Elymus glaucus* Buckl., *E. interruptus* Buckl., *E. junceus* Fisch., *Festuca rubra* var. *commutata* Gaud., *Hordeum vulgare* L., *Panicum miliaceum* L., *Secale cereale* L., *Setaria italica* (L.) Beauv., *S. viridis* (L.) Beauv., *Stipa comata* Trin. and Rupr., *Triticum aestivum* L., *T. dicoccum* Schrank, *T. durum* Desf. and *Zea mays* L. Under the same conditions of experimentation, many of the sphaerosporangial forms obtained from damping-off grasses proved to be slightly, and less often moderately, parasitic on wheat, while others showed no signs of parasitism.

In addition to the hosts listed above, the following species have yielded *Pythium arrhenomanes* from diseased field-grown, naturally infected plants, but the parasitic nature of these isolates remains to be proved: *Agropyron desertorum* (Link) Richt., *A. riparium* Scribn. and Sm., *A. spicatum* (Pursh) Scribn. and Sm., *A. trichophorum* (Link) Richt., *Arrhenatherum elatius* (L.) Mert. and Koch, *Avena fatua* L., *A. sativa* L., *Sorghastrum nutans* (L). Nash, *Sorghum vulgare* Pers., and *S. vulgare* var. *sudanense* (Piper) Hitch. Since all of the isolates of *P. arrhenomanes* tested on wheat were definitely pathogenic, it is believed that most or all of the untested cultures of this fungus will prove similarly so.

It would appear from the foregoing results that damage to cereals and grasses from *Pythium* is just as common and serious in the Northern Great Plains as in Saskatchewan and that, as has been previously pointed out, the parasitic species concerned are indigenous on our native grasses.

A more detailed host-range and list of isolates, together with data on other species of *Pythium* besides *P. arrhenomanes*, will be assembled later.—T. C. VANTERPOOL, University of Saskatchewan, Saskatoon, Canada, and RODERICK SPRAGUE, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Northern Great Plains Field Station, Mandan, N. Dak.

Savoy Disease of Sugar Beets in Southwestern Ontario.[1]—In the course of periodic surveys of sugar-beet fields of southwestern Ontario during the current season, some hundreds of plants exhibiting symptoms suggestive of disease of the virus type were marked for special observation. In the case of certain of the suspect plants the cause and nature of their apparently diseased condition remain obscure. In the case of others, however, the symptoms exhibited were identical in every respect with those described by Coons and co-workers[2] in 1937, for Savoy, a virus disease of beets transmitted by the pigweed bug, *Piesma cinerea*. The latter insect, which has a wide host range including the red root pigweed and other members of the pigweed family, has been reported by Stirrett[3] as occurring in the sugar-beet fields in Ontario. Figure 1 shows a severe case of Savoy, infection

[1] Contribution No. 686 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Coons, G. H., J. E. Kotila and Dewey Stewart. Savoy, a virus disease of beet transmitted by *Piesma cinerea*. Phytopath. (Abstract) **27**: 125. 1937.

[3] Stirrett, Geo. M. A contribution to the knowledge of sugar beet insects in Ontario. Sci. Agr. **16**: 180–196. 1935.

having reached the stage of total involvement of the leaves of a 5-month-old sugar beet.

FIG. 1. Five-month-old sugar beet showing typical, late-stage symptoms of Savoy disease. $\times \frac{2}{3}$.

As far as the writers are aware, this disease of beets has not been reported heretofore in Ontario, but it is known that in October, 1939, H. D.

Brown of the Agricultural Research Department of the Canada and Dominion Sugar Company, Chatham, Ontario, had "Savoy" plants under observation.

In most of the fields examined this year only a trace of the disease was found. However, in several fields in one particular district, the number of affected plants reached proportions that must have been of appreciable economic significance. The circumstances in connection with the incidence of affected plants in one of these fields seem of sufficient interest to warrant consideration in the following detail. Commencing with the outside row on one side of the field and proceeding row by row towards the center, 11,200 plants were counted in successive groups of 100 within each row (Fig. 2).

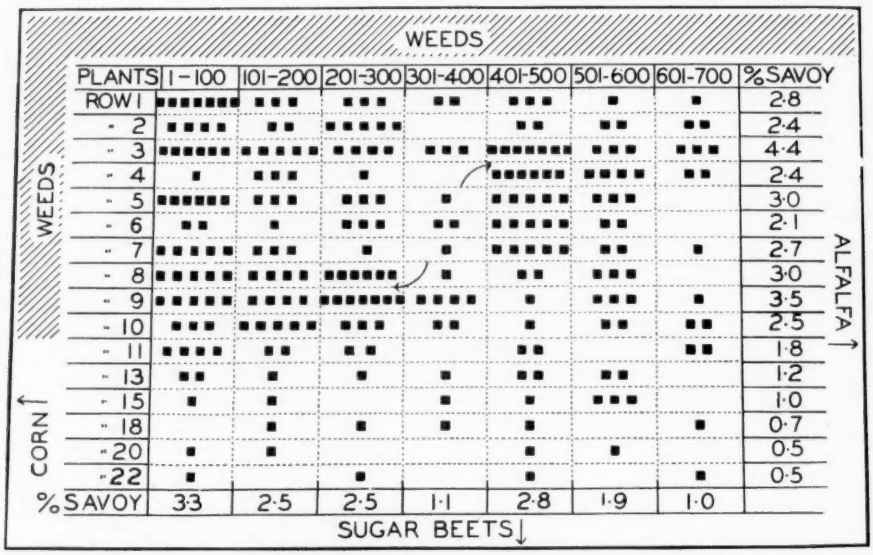

FIG. 2. Diagram showing correlation between occurrence of sugar beets infected with Savoy disease and presence of weeds in close proximity.

Altogether, 245 plants, that is, 2.1 per cent, showed definite symptoms of Savoy. Analysis of the data of the completed count indicated that infected plants were not distributed evenly throughout the field. Rather, it was noted that infection was greater in one corner and along one side of the field than towards the center. For example, the percentage occurrence of the disease in rows 3, 11, and 22, was 4.4, 1.8, and 0.5, respectively. In the first 10 rows the number of infected plants averaged 2.6 per cent, whereas in the next 6 rows counted (11, 13, 15, 18, 20, and 22), the average dropped to 0.9 per cent. In the areas indicated by the arrows in figure 2, infection reached a maximum of 6.5 per cent.

Along the one side and at the corner of the field towards which infection was highest, weeds were growing in profusion. Among the latter *Ambrosia artemisiifolia* greatly predominated, but there were also present *Daucus carota*, *Solidago canadensis*, *Chenopodium album*, *Trifolium repens*,

Amaranthus spp. and others in lesser numbers. Circumstantial evidence would suggest that some of the weeds mentioned above were infected with the virus transmitted to the beets following invasion of the field by viruliferous insects.—A. A. HILDEBRAND and L. W. KOCH, Laboratory of Plant Pathology, Harrow, Ontario.

Mosaic of Bromus inermis.—During the spring of 1941, the writers collected a yellow mosaic on *Bromus inermis* Leyss. growing in the headland of the wheat rust nursery at the Kansas Agricultural Experiment Station, Manhattan, Kansas. In a limited survey in the vicinity of Manhattan, no other case of mosaic on *B. inermis* was found.

Infected plants from Manhattan were transported to Arlington Farm, Virginia, and inoculation studies were carried out. *Bromus inermis,* Harvest Queen wheat, and White Tartar oats were found susceptible, developing severe yellow-mosaic symptoms. Infected tissue was clipped, dried, and stored at summer room-temperatures and, at intervals, tested for potency in wheat. At the end of 51 days, when the last test was made, there was sufficient active virus to induce mosaic in all of the 12 wheat plants inoculated. Further tests may show the period of potency to be still longer.

Bromus inermis was inoculated with each of the 7 wheat viruses previously listed,[1] and the Bromus virus was used as a control on the technique. Each virus was inoculated into 20 healthy plants of *B. inermis.* The Bromus virus induced typical mosaic in 16 plants, but none of the wheat viruses induced symptoms. Inoculations from these symptomless plants have failed thus far to indicate any virus carriers. It is concluded that the Bromus virus is distinct from the other viruses tested.—H. H. MCKINNEY, H. FELLOWS, and C. O. JOHNSTON, Bureau of Plant Industry, U. S. Department of Agriculture.

A Convenient Scale for Use in the Rapid Determination of Comparative Degrees of Infection of Hops by the Downy Mildew Fungus, Pseudoperonospora humuli.[1]—Moist chambers are prepared, in the course of laboratory inoculation experiments, by placing moistened discs of filter paper in the bottoms of Petri dishes. Excised leaves of hop (*Humulus lupulus* L.) are placed on the filter paper, as shown in figure 1, and distilled water suspensions of the zoosporangia of the downy mildew fungus (*Pseudoperonospora humuli* (Miyabe and Tak.) Wilson) are atomized, with a DeVilbiss atomizer, onto the exposed lower leaf surfaces. The covers are placed on the dishes and the moist chambers then removed to an incubation cabinet maintaining a constant optimum germination temperature of approximately 65° F. After an incubation period of suitable length infection of the leaves

[1] McKinney, H. H. Mosaic diseases of wheat and related cereals. U. S. Dept. of Agric. Cir. No. 442, 22 p. 1937.

[1] Published as Technical Paper No. 405 with the approval of the Director, Oregon Agricultural Experiment Station—Reporting a cooperative project between the U. S. Bureau of Plant Industry and the Oregon Agricultural Experiment Station.

FIG. 1. A Petri-dish moist chamber with cover removed to show hop leaf in place.

FIG. 2. Typical secondary leaf infection resulting from natural infection in the field, of Late Clusters, a susceptible variety of hops.

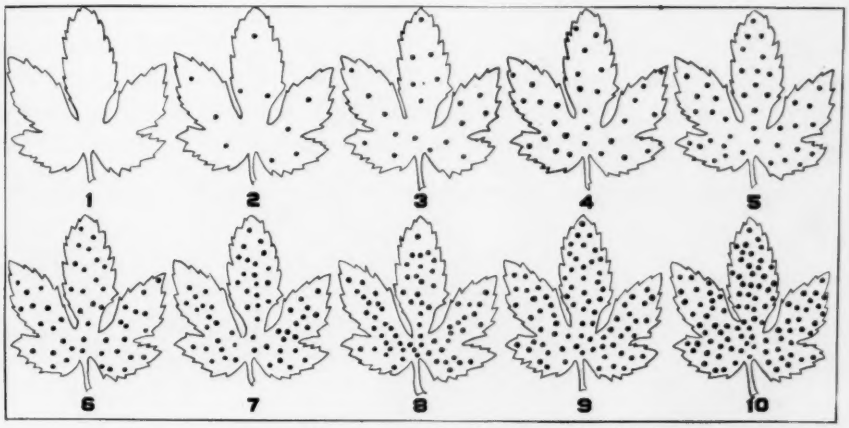

FIG. 3. Scale showing comparative degrees of leaf infection.

of susceptible hosts is evidenced by the appearance of clusters of sporangiophores bearing dark-colored sporangia as shown in figure 2.

The scale found to be convenient for use in the rapid determination of comparative degrees of infection is illustrated in figure 3. It was prepared by tracing the outlines of a hop leaf of convenient size to fit into the Petri dish moist chamber and by placing within each outline a definite number of dots, from 0 to 100, to represent points of infection. Numerals from 0 to 10 are used to represent the comparative degrees of infection. By reference to the scale numerical values can rapidly be assigned to the infection appearing on the leaves as they are removed from the moist chambers, particularly if the leaves are examined with the aid of a watchmaker's loupe.—G. R. HOERNER, Division of Drug and Related Plants, Bureau of Plant Industry, United States Department of Agriculture, Oregon State College, Corvallis, Oregon.

The Rasp Leaf of Cherry.[1]—A disease of cherry trees manifesting itself by abnormalities of the leaf growth was observed first in 1935 on the Royal Ann variety at Cedaredge in Delta County, Colorado. In 1938 cherry trees of the Bing, Lambert, and Royal Ann varieties near Paonia, Delta County, were found affected with the malady. Observations in 1940 and 1941 indicated a quite rapid spread of the disease in this district. The Royal Ann variety appeared to be most severely affected.

The enations, which develop on the underside of the leaves, constitute the most characteristic symptom of the disease and vary from elongated protuberances to raised, serrate, leaf-like growths (Fig. 1, A, D). Usually a gland is present at the apex of each leaf-like growth (Fig. 1, D). On a given leaf the growths usually are found spreading outwardly from the midrib between the veins toward the margin of the leaf blade. The upper surface of the leaf blade shows depressed rugosities, of lighter color than the

[1] Published with the approval of the Director, Colorado Agricultural Experiment Station, as Scientific Series paper #131.

normal green of the leaf (Fig. 1, C). In all cases such ventral depressed and discolored areas are directly opposite a dorsal outgrowth. Because the majority of the dorsal outgrowths resemble the teeth of a coarse rasp and because this is the major manifestation of the disease, the name rasp-leaf is suggested to designate the disease. Severely affected leaves are small, nar-

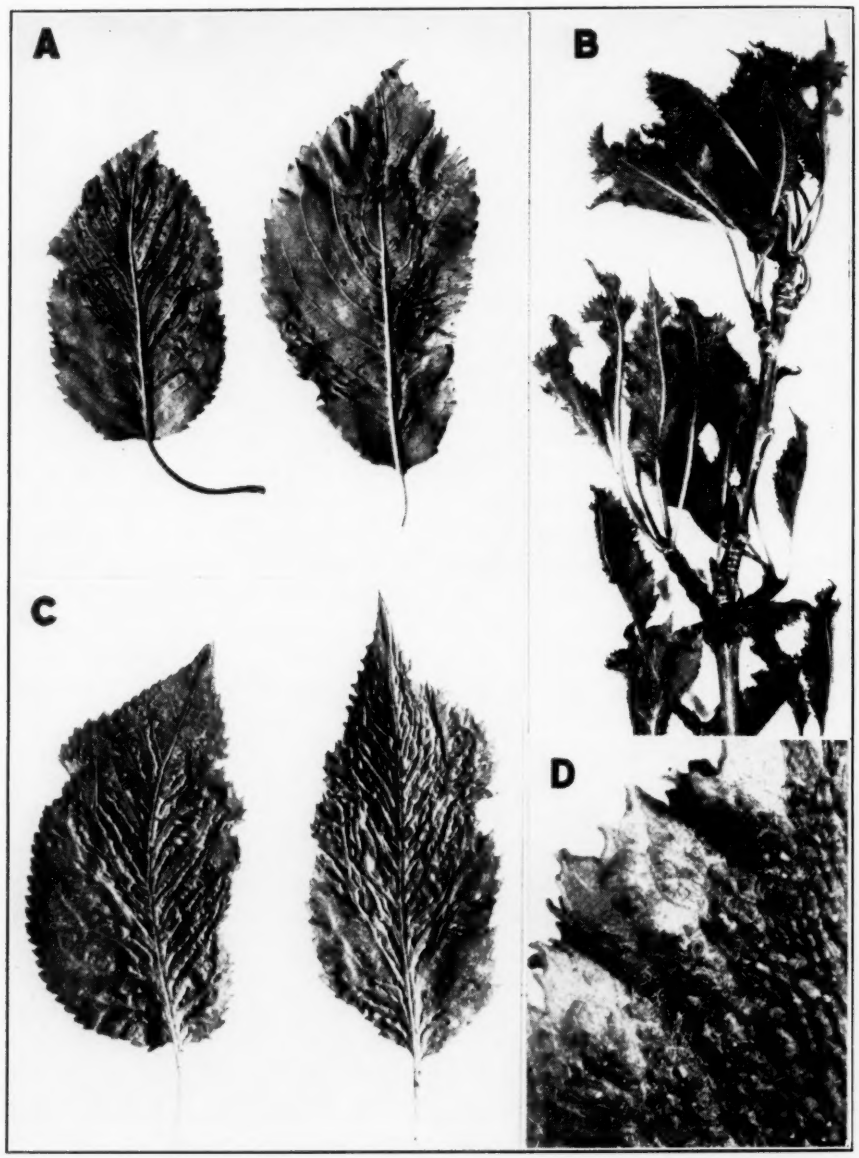

FIG. 1. Symptoms of rasp leaf of cherry on the Royal Ann variety. A. Enations on lower surface of affected leaves. B. Diseased twig showing distortion and ventral folding of the leaves. C. Ventral surface of affected leaves showing depressed rugose areas. D. Enlarged portion of dorsal surface of diseased leaf showing outgrowths. In many instances an apical gland may be noticed.

row, and markedly distorted. The leaf blade frequently shows a tendency to fold upon itself ventrally (Fig. 1, B). The damage to the tree apparently results from the retardation in growth.

Bud-inoculation tests to date indicate that the disease is of a virus nature, with a two-year incubation period.—E. W. BODINE, Section of Botany and Plant Pathology, Colorado Experiment Station, and J. H. NEWTON, Bureau of Plant and Insect Control, Colorado State Division of Agriculture.

The Ascogenous Stage of the Peach Constriction-disease Pathogen.—In PHYTOPATHOLOGY **30**, pages 966 and 967, the writer set forth reasons for considering the species of *Phomopsis* causing peach constriction-disease as a conidial stage of *Diaporthe eres* in the sense of Wehmeyer, or *D. perniciosa* in the sense of certain European investigators, although he had not found

FIG. 1. Asci and ascospores of *Diaporthe* from culture of peach *Phomopsis*. ×950. Mounted and photographed by Marguerite Wilcox.

an ascogenous stage either occurring naturally or in cultures. Recently perithecia were found in a culture of the peach Phomopsis from Delaware, which, in the above mentioned publication, was called "Peach III." A culture of "Peach III" was transferred to oatmeal agar on Dec. 4, 1940. Five days later a small piece of peach twig, sterilized by flaming, was placed on this vigorously growing culture. The fungus continued to grow vigorously, and in one month produced typical pycnidia on both the agar and the twig. The culture was not again examined until September, 1941, when perithecia were found on both the agar and the twig. These perithecia were

nearly spherical with short ostioles projecting from the substrate and were typical for the genus *Diaporthe*. Paraphyses were present. The asci were clavate with a refractive ring in the apical wall. The ascospores typically were uniseptate, constricted at the septum, and 4 guttate, although when slightly out of focus they appeared triseptate. Asci measured 49–57 × 8–10 μ; ascospores 12–13 × 4 μ, biseriately arranged (Fig. 1). Attempts to germinate ascospores were unsuccessful, probably because the culture was so old and dry. The morphological characters and measurements of the perithecia, asci, and ascospores support the view that the fungus is *D. eres* in the sense of Wehmeyer or *D. perniciosa* in the sense of many European investigators, and for the present it is so considered by the writer. However, any one working with the various forms of *Phomopsis* occurring on deciduous fruit trees must realize the need for a more thorough investigation of these forms and their ascogenous stages before taxonomic relationships can be determined with certainty.—JOHN W. ROBERTS, Principal Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Beltsville, Maryland.

Poinsettia Scab Discovered in Honolulu.—In December, 1940, E. A. Bessey informed the writer of the discovery of a scabbed condition of a double variety of poinsettia (*Euphorbia pulcherrima* Willd.) in Honolulu, T. H., during his recent stay there. The disease was found in November, 1939, by E. C. Zimmerman, Entomologist of the Bishop Museum. It was discovered in a nursery where, as Dr. Bessey stated, the large number of

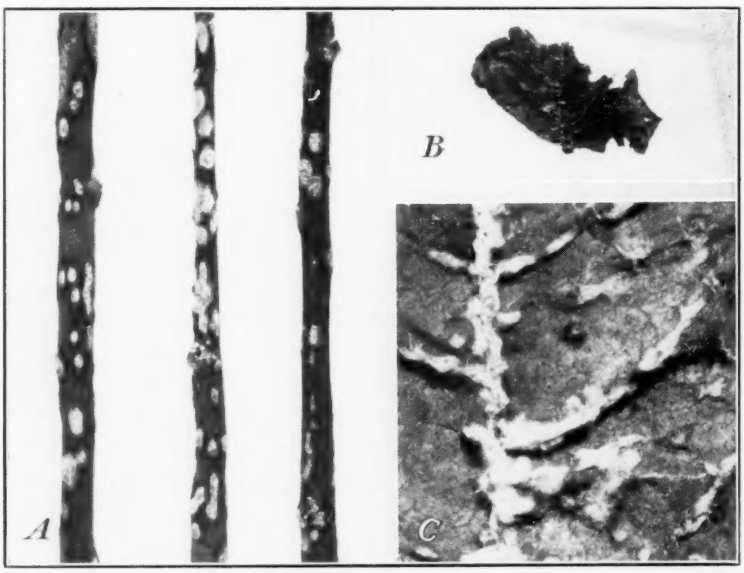

FIG. 1. Poinsettia scab from Honolulu. *A*. Lesion on stems. × 1. *B*. Part of a leaf showing infection principally on midrib and veins. × 1. *C*. Part of *B*. × 8. Photographs by M. L. F. Foubert.

poinsettias grown were much damaged by the attack. From specimens he contributed to the writer early in 1941 (Fig. 1), his preliminary identification of the fungus present as *Sphaceloma* was verified. A pure culture, which he isolated from the scab lesions, as he described it, was probably of the *Sphaceloma*. Dr. Bessey also sent a specimen of the same or a similar disease of *Pedilanthus* sp. This had been collected in a private garden in Honolulu, by the owner, also in November, 1939. In this case further diagnosis awaits additional material. The scab of poinsettia from Honolulu appears to be identical with that found in southern Florida in July, 1940, and subsequently, as recently reported by Ruehle,[1] from whom a specimen is at hand.—ANNA E. JENKINS, Bureau of Plant Industry, Washington, D. C.

[1] Ruehle, G. D. Poinsettia scab caused by *Sphaceloma*. Phytopath. 31: 947–948. 1941.

REPORT OF THE THIRTY-THIRD ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

THE 1941 DALLAS MEETING

The thirty-third annual meeting of The American Phytopathological Society was held in Dallas, Texas, from December 29, 1941, to January 1, 1942. While the attendance did not equal that of some former years, the meeting was most timely and successful. Seventy-five new members were elected at Dallas, which now gives the Society a membership of 1118.

Approximately 170 attended the Phytopathologists' Dinner at the Hotel Adolphus, and enjoyed the typical southwestern entertainment arranged under the chairmanship of K. Starr Chester, with L. M. Blank and W. H. Tharp assisting.

Special conferences were held on virus diseases from the plant quarantine standpoint, results of cooperative seed-treatment experiments, and plant pathology in relation to national defense and post-war readjustments. The invitation program of demonstrations, under the efficient direction of A. J. Riker, chairman, P. A. Young and E. W. Lyle, again proved its usefulness and was highly commended. The value of cooperation among different branches of plant science was brought out in joint sessions with Section G, of the A.A.A.S., and affiliated botanical societies, the Potato Association of America, and the Mycological Society of America.

The climax of the meeting came with the appointment of a War Emergency Committee, E. C. Stakman, J. G. Leach, and R. P. White, with the power to expand and include members from the five geographical divisions of the country.

The matter of place and date of the 1942 summer meeting will be announced later by the program committee.

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International Union of Biological Sciences, A. G. NEWHALL.

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Union of American Biological Societies (and Biological Abstracts), H. B. HUMPHREY and C. C. ALLISON (*ex officio*), F. V. RAND, C. W. BENNETT, H. A. EDSON, G. W. KEITT.

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Fungus Nomenclature, to Work with British Section of Mycological Society. G. L. ZUNDEL, Chm., C. M. TUCKER, J. A. STEVENSON, D. S. WELCH, ERDMAN WEST.

International Cooperation and Reorganization. L. M. HUTCHINS, Chm., CHARLES CHUPP, W. J. ZAUMEYER, FREEMAN WEISS, J. J. CHRISTENSEN, J. C. WALKER, J. A. STEVENSON, C. W. BENNETT.

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Seed Certification. J. C. WALKER, Chm., E. J. WELLHAUSEN, S. J. P. CHILTON, S. M. ZELLER, W. D. MOORE.

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War Emergency. E. C. STAKMAN, J. G. LEACH, R. P. WHITE.

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Resolutions, O. D. BURKE, G. H. GODFREY, JAMES G. BROWN.

REPORT OF OFFICERS, REPRESENTATIVES, AND COMMITTEES FOR 1941

Report of the Secretary. The society year 1941 opened with 1128 members and closed with 1118, a net loss of 10 members. At the Dallas meeting 75 new members were elected. Six former members were restored to the active roll during the year. The society lost 91 members, 16 by resignation, 4 by death, and 71 by suspension for nonpayment of dues. It might be of interest to the membership to know that, of the 71 suspended, 2 were from South America, 3 from Canada, and 23 from other foreign countries, a total of almost half of those suspended, although the foreign membership is comparatively small. Of the full membership 154 are paid-up life members and two are paying $10.00 a year toward life membership.

The Society's clearing agency, to facilitate contact between employable plant pathologists and pathological openings from individuals or institutions, was continued in 1941. Applications were received or on hand from 55 pathologists. Sixty-six applications from 38 pathologists were sent to 15 prospective employers. It was reported to the agency that three pathologists were hired through contact first made through the agency.

The secretary wishes to thank the members and officers of the Society for their help and cooperation during his four years' service. Special thanks are given to Mrs. O. D. Burke for her painstaking and unselfish secretarial assistance.

Report of the Treasurer. Statement of accounts for the year ending November 30, 1941.

Receipts:

Balance from 1940			$2103.71
Annual dues:			
1939	$ 6.68		
1940	26.99		
1941	2870.60	($10.00, life)	
1942	1958.34	(20.00, life)	
1943	12.50		
1944	10.00		
1945	10.00		
1946	5.00	$4900.11	
Voluntary dues		10.00	
Donations from members for foreign subscriptions		85.00	
Balance from A. P. S. dinner in Philadelphia		20.60	
Index payments included with checks for dues		335.00	
Reimbursement for checks returned by bank		10.00	
Total receipts			5360.71
			$7464.42

Expenditures:

Member subscriptions transferred to PHYTOPATHOLOGY:			
1939	$ 5.68		
1940	20.51		
1941	3667.25	$3693.44	
Transferred to Sinking Fund (Building and Loan)		15.00	
Transferred to PHYTOPATHOLOGY for:			
30-Year Index	278.00		
Donations for foreign subscriptions	85.00		
Voluntary dues	10.00	373.00	
Secretarial work and expenses of office of Secretary		236.58	
Secretarial work for Treasurer		297.50	
Preprints of abstracts		35.46	
Reprints of Constitution		20.88	
Printing		139.44	
Stamps and stamped envelopes		41.03	
Supplies		1.45	
Demonstration programs:			
1940	12.50		
1941	7.41	19.91	
Binding 4 volumes of official set of PHYTOPATHOLOGY		7.60	
Addressing bills		1.11	
Dues refunded		1.50	
Checks returned by bank		10.00	
Collection of checks		1.05	
Total expenditures			4894.95
Balance on hand			2569.47
			$7464.42

Sinking Fund. The Sinking Fund, the income from which is used for the support of PHYTOPATHOLOGY, has been obtained by deducting $5.00 from each life-sustaining membership installment. All but two of the life-sustaining members have paid in full. As one of these is in England and the other in Italy, it will probably be several years before they can complete their payments. The fund totaled $9646.00 at the close of 1940. During the year it increased to $9661.00 and is invested as follows:

First mortgage notes deposited with the McLachlen Banking Corporation for collection ($10000.00 at 6%, $500.00 at 5%)	$1500.00
Invested with the following building and loan associations:	
Arlington and Fairfax Bldg. and Loan, 5%	1000.00
Columbia Permanent Bldg. Association, 4%	510.00
District Bldg. and Loan Association, 4%	1656.08
National Permanent Bldg. Association, 4½%	2000.00
Northwestern Fed. Savings and Loan Association, 3½%	2000.00
Perpetual Bldg. Association, 4%	1020.54
Prudential Bldg. Association, 4% (accrued interest, $2.92)	163.92
	$9850.54
Less interest due PHYTOPATHOLOGY	189.54
	$9661.00

The Lyman Memorial Fund, obtained from voluntary contributions, now totals $3181.53, of which $54.21 represents accrued interest and is available for PHYTOPATHOLOGY. The whole amount is invested with the Brookland Building and Loan Association at 3½%. The account for 1941 follows:

Balance on hand Dec. 15, 1941	$3264.66
Dividends, Dec. 31, 1940, and June 30, 1941	111.16
Contributions from members	20.25
Sales of Erwin F. Smith Memoir	15.00
	3411.07
Accrued dividends, transferred to PHYTOPATHOLOGY, Jan., 1941	229.54
	$3181.53

Report of the Business Manager of PHYTOPATHOLOGY. At the close of 1940 there were 600 nonmember subscribers, including 5 complimentary. During 1941 there were 28 cancellations and 65 suspensions, a loss of 93. This more than offset the 77 new subscriptions and resulted in a net loss of 16, bringing the list at the close of 1941 down to 584, the lowest since 1935. Even this figure will be materially reduced beginning with 1942, when, due to the loss of foreign subscriptions, the list will fall at once to less than 500 with a probability that there will be a further drop of 50 to 100.

Statement of accounts for the year ending November 30, 1941.

Receipts:

Balance from 1940			$ 7288.35
Subscriptions:			
1940	$ 97.10		
1941	2957.19		
1942	381.34		
1943	11.50	$3447.13	
Member subscriptions:			
1939	5.68		
1940	20.51		
1941	3667.25	3693.44	
Sales of back numbers		330.25	
Advertising:			
1940	125.16		
1941	628.74	753.90	
Interest on Sinking Fund:			
First mortgages	85.00		
Building and Loan	454.24	539.24	
Grant from Rockefeller Institute		600.00	
Interest on current funds		173.16	
Interest on Lyman Fund		229.54	
Allowance on reprints		557.70	
From authors for excess illustrations		244.80	
Part payment by National Academy of Science for article in March issue		60.00	
30-Year Index		2040.75	
From A. P. S. for:			
Foreign subscriptions donated by members	85.00		
Voluntary dues	10.00	95.00	
Items for other accounts included with checks for subscriptions and Index:			
E. F. Smith Memoir	1.45		
Classics	3.25		
Lyman Fund	5.25	9.95	
Reimbursement for checks returned by bank		23.75	
Total receipts			12798.61
			$20086.96

Expenditures:

Printing, distributing and storing PHYTOPATHOLOGY:		
Vol. XXX, No. 11	$855.71	
No. 12 and Index	798.58	

Vol. XXXI, No. 1	895.50		
No. 2	797.99		
No. 3	655.56		
No. 4	761.16		
No. 5	817.03		
No. 6	795.57		
No. 7	808.29		
No. 8	726.11		
No. 9	740.83		
No. 10	721.61	$9373.94	
Postage		580.36	$9954.30
Secretarial work and office expenses, Editor in Chief			391.91
Secretarial work, Business Manager			385.60
Allowance, Advertising Manager			55.88
Stamps and stamped envelopes			74.72
Supplies			1.20
Printing			34.51
30-Year Index			3171.46
Interest and storage on paper purchased in advance			62.89
Transferred to other accounts:			
E. F. Smith Memoir	1.45		
Classics	3.25		
Lyman Fund	5.25		9.95
Refund, subscriptions and sales			12.66
Checks returned by bank			23.60
Bank service charge			4.95
Total expenditures			$14183.63
Balance on hand:			
Checking account		$1155.17	
Northwestern Fed. Sav's and Loan Association		4748.16	5903.33
			$20086.96

Report of the Auditing Committee for the year ending November 30, 1941. The books of the Treasurer of The American Phytopathological Society and of the Business Manager of PHYTOPATHOLOGY, including accounts of the Society's Sinking Fund and of the Lyman Memorial Fund, have been examined. We have compared receipts and expenditures with bank statements and find them to be complete and satisfactory in every detail.

ROYAL J. HASKELL
WILLIAM W. DIEHL

Dec. 16, 1941.

Report of the Advertising Manager. There were 93 revenue-producing advertisements, occupying 51 pages and consisting of 23 full-page, 42 half-page, and 28 quarter-page insertions. During the year 12 commercial companies used our Journal as advertising medium.

There were 38 nonrevenue-producing advertisements, occupying 24 pages and made up of notices regarding Phytopathological Classics, the Society clearing agency, the Committee on Public Relations, the 30-Year Index and a few others.

Contracts in 1941 totaled $834.26.

AGNES E. MEIER

Report of the Editor in Chief. Volume 31 of PHYTOPATHOLOGY contains, exclusive of the index, 1142 pages of printed matter and illustrations classified as follows: 109 articles, 51 notes, 3 reports of meetings, 7 book reviews, 159 abstracts, 266 text figures, 1 plate, and 2 frontispieces. From December 16, 1940, to December 10, 1941, a total of 195 manuscripts of articles, notes, book reviews and reports of Society meetings were submitted for publication in PHYTOPATHOLOGY. Sixteen of these manuscripts were either recalled by their authors or returned to them as unsuitable for publication in our Journal. There are now in press 46 articles and 103 abstracts. Other manuscripts now on hand (Dec. 10) comprise a total of 754 typewritten pages. It is estimated that there is in hand enough material to fill 770 pages of PHYTOPATHOLOGY. At this time last year the figure stood at 820 pages, a difference of 50 in favor of 1941.

Continued and increased emphasis on the importance, even the necessity, of condensing and otherwise improving manuscripts has made itself felt throughout the past year. There is room for further improvement, and this improvement must be effected by the

contributors themselves. There is scarcely a manuscript submitted for publication in our Journal but that will bear more or less condensation without any impairment of quality or scientific worth. Manuscripts are still coming in without table headings and with inadequately described illustrations. Still others are accompanied by illustrations of such poor quality that, when reproduced, they fail to reveal the details called for in the legends or described in the text. It is time and money wasted to submit any but high-quality photographs printed on glossy Velox paper or its equivalent.

No manuscript, carrying citations to literature, should be submitted without assurance of the correctness of all citations. Too many contributors still leave this job to the editor. The value and excellence of our Journal will be no better, no greater, than the papers upon which it depends for its continuance.

H. B. HUMPHREY

Report of the Manager of Phytopathological Classics for the year 1941. I beg to submit herewith the annual report of my stewardship as Manager of Phytopathological Classics:

Report for the fiscal year beginning December 1, 1940, and ending December 1, 1941

Classics No. 1:	On hand 12–1–40	80	
	Sold during year	15	
	On hand 12–1–41		65
Classics No. 2:	On hand 12–1–40	281	
	Sold during year	14	
	On hand 12–1–41		267
Classics No. 3:	On hand 12–1–40	380	
	Sold during year	18	
	On hand 12–1–41		362
Classics No. 4:	On hand 12–1–40	442	
	Sold during year	17	
	On hand 12–1–41		425
Classics No. 5:	On hand 12–1–40	688	
	Sold during year	16	
	On hand 12–1–41		672
Classics No. 6:	On hand 12–1–40	813	
	Sold during year	20	
	On hand 12–1–41		793

Cash balance on hand 12–1–40	$282.49	
Receipts during year	64.75	
Total		$347.24
Expenditures:		
Postage	$ 2.10	
Total expenditures		2.10
Balance on hand December 1, 1941		$345.14
Due on accounts		$ 8.75

PHYTOPATHOLOGICAL CLASSICS
Number 7

1. CONCERNING THE MOSAIC DISEASE OF TOBACCO
   By Adolph Mayer
2. CONCERNING THE MOSAIC DISEASE OF THE TOBACCO PLANT
   By Dmittri Ivanowskiĭ

3. CONCERNING A CONTAGIUM VIVIUM FLUIDUM AS A CAUSE OF THE SPOT-DISEASE OF TOBACCO LEAVES
By Martinus W. Beijernick
4. ON THE ETIOLOGY OF INFECTIOUS VARIEGATION
By Erwin Baur

Translated from German
by
James Johnson
With a preface and biographical sketches by the translator
Published by
The American Phytopathological Society
1942

Report of the Necrology Committee. During 1941 there were five deaths within our membership as follows:

KARL VON TUBEUF, February 8;
OTIS CHESTER WHIPPLE, February 13;
LEE ELLIS MILES, May 10;
WALLACE BUTLER, August 3; and
R. R. MCLEAN, September 28.

A. G. JOHNSON
M. B. WAITE

Summary of the Report of the Committee on Biological Abstracts and the Union of American Biological Societies. *What the Biological Abstracts Staff Is Doing for the Biologists.*—Against great odds and in spite of the war, the staff of *Biological Abstracts* has completed a notable year of accomplishment. Few realize the magnitude of the task performed over the last four years by a central office staff, which had been reduced in numbers to about one-fourth its normal size, nor is the enormous amount of extra time and nervous energy necessarily expended by the few to do the work of the many fully appreciated. Even in face of these handicaps, however, very conspicuous progress has been made.

The war has had a tremendous effect on scientific output and distribution. Now, as never before, the remaining abstract journals are demonstrating their importance. During 1941, therefore, *Biological Abstracts* has made every consistent effort within its power and means to produce abstracts of as much as possible of the literature that the war has made directly unavailable to most biologists outside the countries of origin. In carrying out these endeavors, the aid of many biologists in foreign countries has been obtained in furnishing abstracts from journals not now available here. *Biological Abstracts*, on its side, is also helping British abstract journals to obtain material not now directly available to them.

Toward the ends enumerated, 1550 serial publications are now (December, 1941) being regularly covered as against 1105 early in the year; and 24,759 entries appear in volume 15 as compared with 17,038 for the preceding year. During the first half of 1941 the indexes for 1940 were completed and mailed, a notable feat in itself. Furthermore, all the preliminary correspondence and organization has been completed for the new sectional edition being launched with the first issue of 1942. In the 1941 volume, *Section D—Abstracts of Plant Sciences* carried 7060 entries from about 951 journals, and phytopathology alone had 1215 entries from about 292 journals. In each case the number of entries was approximately doubled and the journal sources were increased by about one-third over the preceding year. Additional journals as pointed to by cross references would add about 30 per cent to the number of journals covered.

What the Biologists Are Doing for Biological Abstracts.—In the giving of time and effort and moral support the biologists have not been found wanting. From past experience your Chairman knows something of what the eleven members of the Board of Trustees and its Secretary have put into the enterprise. There are also 143 specialists on the Editorial Board, and nineteen biologists participate in the indexing. Well over 1000 individuals prepare abstracts of the papers of other biologists, and several hundred journals are now covered through assignments by their editorial staffs for author abstracts. However, as to financial backing, so sorely needed during these war times, scarcely one biologist out of every eight in the United States is said to be making any contribution at all.

With regard to paid subscription commitments, the United States showed a net gain of 31 for the complete and 93 for the sectional editions over the 1940 record. Foreign subscriptions as a whole showed for 1941 a net loss of 11 for the complete and a net gain of 25 for the sectional editions, and this picture would have been much less favorable had it not been for the approximate doubling of subscriptions among 10 Latin American countries. The total subscription commitments (complete and sectional) for 1941 were

2055 for the United States and 834 for all other countries, making a grand total of 2889 and representing net gains of 20 for the complete and 118 for the sectional editions over 1940. To *Section D—Abstracts of Plant Sciences*, there were only 421 subscribers in 1941! With the partial or complete breakdown of most abstracting services outside the United States, we of America owe it to our own selfish interests, as well as to our abstract journals, to give them fuller financial as well as intellectual support.

Outlook for the Future.—Advancement has been made against odds. The hope is that this momentum may be progressively maintained: its continuance rests largely on increasing gains in support by the individual biologists of the country: the greater this financial support the more adequate will coverage of the literature become. An encouraging sign is the increasing awareness by biologists of the United States that *Biological Abstracts* is their own instrument. This is evidenced not only by growing individual interest, but also by a very considerable financial support accorded through some eight national societies with biological proclivities. What then of the future? It is the firm resolution of the responsible staff that year by year *Biological Abstracts* shall be made more complete, prompt, and effective, and in all respects more useful. Judging from the present outlook, the foreign income will be less and the expenses greater during the year ahead. If, therefore, the current prompt and increasingly adequate coverage is to be maintained, the whole-hearted support and cooperation of the biological societies and individual biologists of this hemisphere will be urgently needed, nay indispensable.

At such a time as this our professional group can make an important contribution by increased efforts to insure the maintenance of *Biological Abstracts* at the highest possible level of efficiency. Your committee suggests that each society member do what he can toward this end by helping to augment the number of American subscriptions. The results will be particularly valuable at a time when every resource of science must be made promptly available for National Defense.

FREDERICK V. RAND, Chairman

December 16, 1941.

Report of the Extension Work and Relations Committee. The Extension Work and Relations Committee sponsored one activity in 1941. This was a panel discussion on the subject: "Plant Pathology in relation to national defense and post-war readjustments" on December 30 at the Dallas Meeting. A detailed report of this conference will appear in the Extension Pathologist in the near future. At the same session the committee sponsored the showing of kodachrome film depicting the "Speedsprayer."

C. C. ALLISON, Chairman, O. D. BURKE, R. J. HASKELL, G. W. KEITT, R. H. PORTER, OTTO REINKING, R. C. ROSE, D. R. SANDS, W. B. TISDALE.

Report of the Committee for Coordination of Research in Cereal and Vegetable Seed Treatments. The activities of this committee for the past year will be presented under the following subdivisions: vegetables, cotton, cereals, and flax.

Vegetable Seed Treatments. Under the general chairmanship of Harold T. Cook, cooperative seed treatment tests were conducted by thirty-eight cooperators located at thirty-one stations in twenty-five states and in two provinces of Canada. C. M. Haenseler personally supervised the lettuce and sweet corn tests; L. D. Leach, the celery tests; G. L. McNew, the cabbage and beet tests; J. C. Walker—not a member of the committee—the cucumber and pea tests; and Harold T. Cook, the spinach tests.

Summaries of this work will be presented by these supervisors at these meetings, and the detailed results have been mimeographed for distribution to the cooperators and others interested. Although the results of tests in 1940 were mimeographed and distributed at the 1940 meetings, the entire report has since been published in the Extension Pathologist for February, 1941.

It seems desirable to continue the vegetable work, possibly on a somewhat reduced scale, for at least a few more seasons.

Cotton Seed Treatments. The work on cotton seed treatments has been carried on for several years under the auspices of the Cotton Seedling Disease Committee of the Cotton Disease Council and under the direct supervision of C. H. Arndt.

Until January, 1941, when Dr. Arndt was placed on the Cereal and Vegetable Seed Treatment Committee, there was no formal association of the two committee. Since that time, the Cotton Seedling Disease Committee has continued its program, and Dr. Arndt has prepared a special summary of six years of experimental studies of cotton seed treatments. This summary is mimeographed and will be distributed to those interested.

Cereals and Flax. In this field, tests were made to determine the effectiveness of various fungicides in increasing yields of wheat, oats, barley, and flax. Other tests were made to determine more directly the effectiveness of these fungicides in controlling smuts of wheat and oats, and barley stripe. These two series of tests were carried on at nine of the more northern States and three Canadian Provinces.

In addition, a new phase of work dealing with corn seed treatment was undertaken for the first time this year and was placed at six different stations. In these tests, only seedling data were taken, since it is impossible to secure varieties which are adapted in length of the growing season to the various states. While the results this year indicate no very pronounced differences in seedling stand, it is thought desirable to continue this work with possibly some modifications of the present plan. It would be highly desirable to have someone from one of the corn belt states placed on this committee to take full charge of the project.

A complete summary of the results of all these tests for the past three years is being prepared and will be distributed before the next crop season.

At the present time, no work is being done on cereal seed treatments in either the hard red or soft red winter wheat areas or in any of the southern states. It would seem highly desirable that such work should be undertaken; and since the problems and the cooperating stations are distinct from those concerned in the more northern states, it would be desirable to have this undertaken by people located in those areas.

M. B. MOORE, Chairman

Report of the Committee on Standardization of Fungicidal Tests. A joint meeting of your Committee with the American Association of Economic Entomologists' Committee on Insecticide Terminology was held at the time of the Philadelphia convention and a number of problems of mutual interest discussed. The two Committees agreed to cooperate wherever possible in formulating standard methods and terminology. Another joint meeting is planned for the New York Convention in 1942–43.

The preparation of tentative "Standard Methods" or lists of "Standard Terms" has been continued and three more are now available in mimeographed form. In each case these have been prepared by the member or members of the Committee especially qualified in cooperation with other specialists.

The new tentative standards are:

1. Tentative Definitions of Fungicide Terms.
2. Tentative Standardized Laboratory and Greenhouse Procedure for Testing the Relative Fungicidal Efficiency of Chemical Dusts in the Control of Certain Cereal Smuts.
3. Tentative Recommendations on Standard Spray Nomenclature (Part II. Pears and Cherries).

Copies of these may be obtained from the Committee Chairman, Boyce Thompson Institute, Yonkers, New York. Copies are also available for the four methods prepared earlier, namely:

1. Tentative Method on a Standard Bordeaux Mixture for Laboratory Tests and for Determination of Bordeaux Coefficient.
2. Tentative Method for Determination of Mean Particle Diameter of Fungicides.
3. Tentative Speciafications for Slide-Moist Chamber Method of Testing Protective Fungicides.
4. Tentative Recommendations on Standard Spray Nomenclature (Part I. Apples and Peaches).

The Committee again requests that these various methods and terms be subjected to trial and constructive criticisms. Also that suggestions be made regarding new methods and terms suitable for standardization. Your cooperation is especially invited, since, shortly, sufficient time will have elapsed to take action on some of these methods as to (1) adaption by the Society as a Standard Method, (2) modification for further consideration, or (3) discarding.

S. E. A. MCCALLAN, Chairman, K. J. KADOW, J. G. HORSFALL, R. W. LEUKEL, J. W. ROBERTS, C. F. TAYLOR, J. D. WILSON.

Report of the Advisory Committee for 1941. During the past year a number of proposals and suggestions were submitted to the Advisory Committee on Society Activities and Programs, which were given attention by its members. From the comments received, it was clearly indicated that the programs at present are being conducted in a very satisfactory manner and no general criticism is to be offered.

The following points were considered by the Committee and are suggested to the Council:

1. That a special or standing committee on "Constitutional Revisions and Amendments" be appointed whose duty it would be to initiate matters of this type and weigh suggestions. It is believed that this should not be the function of the Advisory Committee.
2. To avoid triple sessions at the winter meetings wherever possible.
3. Not to feature symposia to the extent of not having any other session at the same time.
4. Since the programs of the Society are so well conducted at the present time, making it difficult to suggest specific improvements it is proposed by an almost unanimous

vote of the Committee that the Advisory Committee on Society Activities and Programs be discontinued, leaving it to future councils to appoint a special or standing committee as the need arises. It has been suggested that there should be a greater turnover of committees and the holding of them together, although inactive for some hypothetical emergency, is unwise because it adds to the machinery with no effect.

Respectfully submitted,

W. J. ZAUMEYER, Chairman, R. H. DRAYTON, A. A. DUNLAP,
J. A. PINCKARD, R. K. VOORHEES, J. C. WALKER, C. E. YARWOOD.

Report of the Committee on Public Relations. During the past year your committee again followed the objectives outlined to the Society at its 1939 meeting (Phytopath. 30: 368). Since this meeting, international events have focused our attention upon increased agricultural production. As a result of these changes, your committee has endeavored to give to the public as much information as possible on the new developments in our science. Every newsworthy article appearing in Volume 31 of PHYTOPATHOLOGY has been submitted to one or more selected news agencies for national distribution.

It is the belief of your committee that even greater efforts should be made to inform the public of the latest developments in plant disease control. That Food for Freedom shall not fail, each member of the Society is personally responsible to the American Public for this information.

For your convenience in fulfilling your responsibilities a list of accredited science writers and their addresses is appended:

Dr. Gobind Behari Lal, International News Service, 235 E. 45th St., New York, N. Y.
Mr. Waldemar Kaempffert, 10th Floor, The New York Times, N. Y.
Mr. William L. Laurence, The New York Times, N. Y.
Mr. Tom Ryland, Time, Rockefeller Center, N. Y.
Mr. Steven M. Spencer, The Evening Bulletin, Philadelphia, Pa.
Dr. Robert Potter, American Weekly, 235 E. 45th Street, N. Y.
Mr. John J. O'Neill, Science Editor, New York Herald Tribune. N. Y.
Mr. Stephen J. McDonough, The Associated Press, Star Bldg., Washington, D. C.
Mr. Howard W. Blakeslee, The Associated Press, 50 Rockefeller Plaza, New York, N. Y.
Mr. Watson Davis, Science Service, 2201 Constitution Ave., Washington, D. C.
Mr. David Dietz, Cleveland Press Bldg., Cleveland, Ohio.
Mr. Gerard Piel, Life, 6 Rockefeller Plaza, New York, N. Y.
Mr. Thomas R. Henry, The Washington Star, Washington, D. C.
Mr. John R. Pfeiffer, Newsweek, RKO Building, Rockefeller Center, New York, N. Y.
Mr. Allen Schoenfeld, Ann Arbor Bureau, Ann Arbor, Mich.
Mr. Albert E. Wiggam, 241 Central Park West, New York, N. Y.
Mr. John White, Washington Times Herald, Washington, D. C.
Mr. Henry Platt, Editor, Predate, 220 East 42nd Street, New York, N. Y.
Miss Harriet Mackintosh, Fairchild Publications, 8 E. 13th St., New York, N. Y.
Mr. Max Gilstrap, The Christian Science Monitor, Boston, Mass.
Special Service Bureau, United Press, Daily News Bldg., New York, N. Y.

Your committee wishes to express special thanks to Mr. Will A. Whitney for contributing freely his time in rewriting numerous scientific articles for the press. The committee also wishes to express its gratitude to the Editor in Chief, the printers, and others for their appreciation of our needs and for the assistance they have rendered.

J. A. PINCKARD, Chairman, O. C. BOYD, C. T. GREGORY,
J. H. JENSEN, FRANK MCWHORTER, A. G. NEWHALL,
G. H. STARR, A. J. ULLSTRUP, G. F. WEBER, P. A. YOUNG

Report of the Committee on Regulatory Work and Foreign Plant Diseases. The conference on "Virus diseases from the Plant Quarantine Standpoint" was held in Dallas, Texas, as scheduled on December 29, 1941, with about 60 in attendance.

An introductory paper by Eubanks Carsner called attention to a number of foreign virus diseases worthy of consideration from the quarantine viewpoint, and a second paper by S. A. Rohwer discussed the limitations within which plant quarantines would have to work to give effective exclusion of these virus troubles. He further requested the Society to aid the quarantine authorities in the formulation of a corret national policy toward foreign virus diseases, and to recommend any of these for which quarantine action would be justified.

These papers and subsequent discussion brought out several points worthy of mention.

1. It was emphasized that there is a great lack of knowledge on foreign virus diseases and that studies of these by competent pathologists, especially in their native homes, would be a basic need for intelligent quarantine action.

2. Inspection methods have a very limited usefulness as a quarantine procedure, since the presence of a virus in most incoming plant materials cannot be determined either by

examination or by any practical tests now available. The need for developing virus detection methods was stressed.

3. Various types of quarantine detention for imported plant material likely to carry virus infection were mentioned, including growing the plants here under close observation until health was assured, and the possibility of a more effective isolation, such as could be maintained on an isolated island. Tests by cross inoculation would be a useful feature of this procedure.

4. There would be some usefulness in sending our own plants or their varieties to foreign regions to test their reaction to specific virus diseases occurring abroad, and not yet introduced here.

5. The difficulty of eliminating a virus from living plants by suitable treatment was recognized, and this valuable possibility in quarantine procedure should be energetically explored to add other treatment methods to the few highly suggestive accomplishments already known in this field.

6. Note was taken of the need for a greater number of workers technically trained in virus diseases for service in quarantine problems.

Recommendation

Mr. Rohwer in his address and in the discussion which followed made it clear that the Bureau of Entomology and Plant Quarantine was calling on the Society for its aid in solving the plant quarantine problems involved in foreign virus diseases by expressing its constructive opinion on two points: (1) What should be the correct national quarantine policy toward foreign virus diseases? And (2) recommendations as to any specific virus host, or plant group, or virus complex for which in the interests of this country quarantine action is regarded as justifiable.

Naturally no adequate answer could be made to these questions in the conference itself, and your committee therefore recommends, as a step toward eventual preparation of such answer as may be possible, that this committee be authorized to canvass those members concerned in virus disease activities on the two points raised, with a view to presenting their comment and opinion at a subsequent meeting of the Society for its consideration.

W. A. MCCUBBIN, Chairman
M. R. HARRIS
A. A. BITANCOURT

Final Report of the Committee on 30-year Index of Phytopathology. The first half of copy for the 30-Year Index of Phytopathology was taken to the printer on May 9th and the remainder on May 16th, 1941. The published index was consigned to the U. S. Mail for subscribers the latter part of September. The Index Committee therefore thankfully relinquishes its official status (and work!) and entrusts its collective self again to the comparative nirvana of the general Society membership.

As to the index itself, it must now stand or fall on its own metaphorical feet. In a task of this size (30,900 author and subject entries, to say nothing of the much larger number of volume-page references) and in spite of all due care, it seems inevitable that some errors must have crept in. The Chairman would appreciate having any such called to his attention for later inclusion in the customary "Errata."

FREDERICK V. RAND, Chairman, H. A. EDSON,
GEORGE L. PELTIER, and N. E. STEVENS

Resolution of Appreciation of Work of F. V. Rand. The Society passed the following resolution and voted that it be published in the report of the 1941 annual meeeting:

> The American Phytopathological Society, assembled in annual meeting at Dallas, Texas, December 29 to 31, 1941, hereby expresses its sincere appreciation to Dr. Frederick V. Rand for his service to the Society in organizing and editing the 30-year Index of our Journal PHYTOPATHOLOGY.
>
> His indefatigable energy and devotion to the task, at great personal sacrifice, have placed the Society and the world at large in debt to him for all time. The achievement is outstanding. No scientific society has issued an analytical index more carefully and critically prepared and edited or more inclusive in its scope and usefulness.
>
> The Society also expresses its appreciation to the members who helped in the preparation of index material for individual volumes under Dr. Rand's direction.

HOWARD P. BARSS, Chairman
E. M. JOHNSON
H. A. EDSON

Resolutions of the Committee on Resolutions. RESOLVED that The American Phytopathological Society express its appreciation to the American Association for the Advancement of Science for the very satisfactory arrangements made by its representa-

tives for the meetings of the several sections of our Society, and particularly for the facilities provided for projection.

RESOLVED that the Society convey to the management and staff of the Hotel Adolphus, and especially to Mr. D. T. Segrest and Miss Harriet Rau, its appreciation for the courteous service extended to our members and for cooperation in providing our officers and committees with unusually commodious quarters for conducting business and scientific programs.

RESOLVED that the Society express to the Committee on Local Arrangements, A. A. Dunlap, chairman, A. L. Harrison, L. M. Hutchins, E. W. Lyle, E. L. LeClerg, G. M. Watkins, and V. H. Young, our gratitude for their fine contributions to the success of the meeting; and to the banquet committee, K. Starr Chester, L. M. Blank, and W. H. Tharp, our appreciation of typical southwestern entertainment.

RESOLVED that the Society commend A. J. Riker, chairman of the demonstrational session, and his aides, P. A. Young and E. W. Lyle, for so efficiently providing facilities for this visual means of presenting scientific facts.

RESOLVED that this Society express its appreciation to R. S. Kirby for his four years of loyal, conscientious, and effective service as the Society's Secretary.

RESOLVED that this Society on behalf of its members express to the officers and members of committees our sincere appreciation for the efforts they have made to promote the interests of the Society throughout the year, and to make this annual meeting instructive and enjoyable.

Respectfully submitted,

O. D. BURKE, Chairman
G. H. GODFREY
JAMES G. BROWN

ACTION BY THE SOCIETY AT THE 1941 DALLAS MEETING

Elections and Appointments. The appointments made, as provided by the Constitution, by the President or the Council since the previous meeting, were approved by the Society in business session. The election committee opened and counted the ballots, and the results were announced to the Society at the banquet. The names of those elected and appointed appear earlier in this report in the list of officers, representatives, and committees. Seventy-five applicants were elected to membership.

The Society confirmed the Council's appointment of the following new members to the Editorial Board of PHYTOPATHOLOGY: George L. Peltier, C. O. Johnston, A. G. Plakidas, and E. S. Schultz.

Reports of Officers, Representatives, and Committees. The reports for the year 1941, as presented on previous pages, were read and accepted.

Revision of Constitution. The Society confirmed the Council's recommendation that Article VII of the Constitution read as follows:

ARTICLE VII

Editors, Committees, and Appointments

The Editors of the official organ of the Society shall be selected by the Council subject to the approval of the Society.

Temporary, Special, and Standing committees may be appointed at the discretion of the Society.

Standing committees, having revolving membership, shall be appointed by the Council to deal with matters pertaining to Society relationships.

Special committees shall be appointed by the Council to deal with plant pathological problems of general interest to the Society. Each special committee shall be continued for such period as in the judgment of the Council may be needed for the accomplishment of its purpose. A final report shall be made at the termination of this period.

Temporary committees shall be appointed by the President to serve during his administration.

Revision of Standing Rule 9. The Society confirmed the Council's recommendation that Standing Rule 9 read as follows:

"The following shall be selected by the Council and approved by the Society: two representatives on the Council of the American Association for the Advancement of Science for a two-year term; one representative on the Division of Biology and Agriculture of the National Research Council for a three-year term; one trustee on the Tropical Plant Research Foundation for a five-year term; and, one member of the Editorial Board of the American Journal of Botany for a three-year term."

It was also recommended and confirmed that the subsistence expenses of our representative *will not* be paid at the meeting point. The ticket and subsistence enroute will be paid by National Research Council.

Revision of Standing Rule 5d. It was recommended by the Council and confirmed by the Society that Standing Rule 5d. be deleted to read as follows:

> "d. *Meetings of.* Whenever The American Phytopathological Society meets within the territory of a Division, the Division shall merge its program with that of the parent Society."

Society Affiliation. The Council recommended and the Society confirmed the affiliation of The American Phytopathological Society with the American Society of Agricultural Sciences.

Committee on New Memberships and Subscriptions. At the request of the committee, the Society confirmed the Council's recommendation that the Committee on New Memberships and Subscriptions be discontinued.

Advisory Committee. At the request of the committee, the Council recommended and the Society voted that the Advisory Committee on Society Activities and Programs be discontinued. Appreciation was expressed of the fine work done.

War Emergency Committee. The Council recommended and the Society confirmed the appointment of a special committee on War Emergency, composed of E. C. Stakman, J. G. Leach, and R. P. White, with power to expand the committee, with the understanding that they will add to their membership one man from each of the five regional groups and as many more as in their judgment would be advisable. *Regional representatives* are: Northeast, J. G. Horsfall; Middle Atlantic, R. S. Kirby; Upper Mississippi Valley, I. E. Melhus; South, George M. Armstrong; Pacific Coast, Max Gardner. *Representative of Industry:* J. F. Adams. *Representatives from U. S. Department of Agriculture:* H. P. Barss, W. A. McCubbin, R. J. Haskell. *Canadian Representative:* F. J. Greaney. Ex-officio members: Lee M. Hutchins and C. C. Allison.

Recognition of Merit Committee. The Society confirmed the Council's recommendation that the incoming President appoint a temporary committee to study the matter of some form of recognition for outstanding papers presented before our Society, and that the papers be considered for the A.A.A.S. prize.

Classification of Committees. The Society confirmed the Council's classification of Society committees in accordance with the newly adopted revision of Article VII of the Constitution.

Demonstration Session. The Society confirmed the Council's recommendation that the demonstration session be continued, and that the rules for presentation of regular papers apply to the demonstration session.

Committee expenditures. The Society approved the Council's recommendation that committee expenditures will be honored by the Treasurer only when previously approved by the Council.

Summer Meeting. The Council recommended and the Society voted that the matter of place and date of a summer meeting be decided by the Program Committee.

The 1942 Annual Meeting. The Society confirmed the Council's recommendation that we plan to meet with the A.A.A.S. next year.

ROBERT REDPATH McLEAN

March 7, 1875–September 28, 1941

Mr. McLean was born in Oakland, California. At the age of ten, he moved with his people to Illinois where his father accepted a pastorate. Ten years later, the family returned to California but soon thereafter Mr. McLean returned to Illinois where he was employed in the Marshall Field Department Store in Chicago.

In 1912, Mr. McLean again returned to California, this time to San Diego, where, after a brief connection with the County Horticultural Department, he was employed in the Farm Service Department of the First National Bank. In 1922, he was appointed County Horticultural Commissioner for San Diego County. Later the designation was changed to County Agricultural Commissioner. This position he held to the time of his death.

He was past president of State (California) Association of County Agricultural Commissioners, a member of the board of directors of the Floral Association of San Diego, and also a member of the Rotary Club, Silver Gate Masonic Lodge, and First Baptist Church of San Diego.

Mr. McLean was an honored member of the community, actively interested in all progressive matters. His pleasing personality will be greatly missed.

LEE ELLIS MILES

September 25, 1890–May 10, 1941

Lee Ellis Miles was graduated from Wabash College in 1914, with the degree of Bachelor of Arts, and in 1920 he received the degree of Doctor of Philosophy from the University of Illinois. He spent the summer of 1940 at Harvard University in special mycological studies.

From 1917 to 1920, Dr. Miles held a fellowship in plant physiology at the University of Illinois and during the summers of 1919 and 1920 he was employed by the Bureau of Plant Industry, U. S. Department of Agriculture. From 1920 to 1922, he served as plant pathologist for the Mississippi State Plant Board. From 1922 to 1927, he was Plant Pathologist at the Alabama Agricultural Experiment Station and Professor of Plant Pathology at the Alabama Polytechnic Institute. In 1927–1928, he was Assistant Plant Pathologist at the Washington Agricultural Experiment Station and Assistant Professor of Plant Pathology at the State College of Washington. From 1928 to the time of his death, he served as Plant Pathologist at the Mississippi Agricultural Experiment Station.

While Dr. Miles had rather wide interests in the field of plant pathology, his most active interests in recent years were with the diseases of cotton. He had an active mind, a forceful yet pleasing personality, and a host of friends.

OTIS CHESTER WHIPPLE

February 18, 1907–February 13, 1941

Otis Chester Whipple attended Le Verne College (Los Angeles), Junior College (Sacramento), and the University of California, was graduated from the latter in 1933 with the degree of Bachelor of Science, and received the degree of Doctor of Philosophy from the University of Wisconsin in 1937.

In 1937, Dr. Whipple was appointed to an instructorship in plant pathology at the University of Wisconsin, which position he held to the time of his death.

Dr. Whipple did notable work on diseases of truck crops. He was a young man of unusual promise as an investigator and his pleasing personality won him friends not only among his colleagues but also among growers, canners, and others with whom he came in contact.

ERRATA, VOLUME 31

Page 7, line 6, *read* results mean that *for* results to.
Page 234, paragraph 2, line 7, *read* these *for* there.
Page 238, footnote, 1, *read* J. E. Flynn *for* J. S. Flynn.
Page 357, line 10, *read aurantii for aurantidi.*
Page 423, table 1, *read Microsiphum pisi Kalt. for Microsiphum pisi Kolt.*
Page 470, footnote 3, *read* 1939 *for* 1929.
Page 505, line 2, *transfer* next-to-last line, p. 504, to end of line 2, p. 505, immediately following the last word, to *read* In an old culture on carrots some of . . .
Page 568, table 2, *read granulatus for granulatis.*
Page 627, line 12, *read* reason *for* region.
Page 673, line 14, *read* first leaf appeared *for* first appeared.
Page 935, citation 16, *read* In press *for* Plant Physiol. **16**: 1–18. 1941.
Page 935, citation 17, *read* Willis *for* Wllis.
Page 1045, 5th line from bottom, *read* (*S. holci*) *for* (Sholci).
Page 1076, line 16, *read* 2.5 *for* 25.